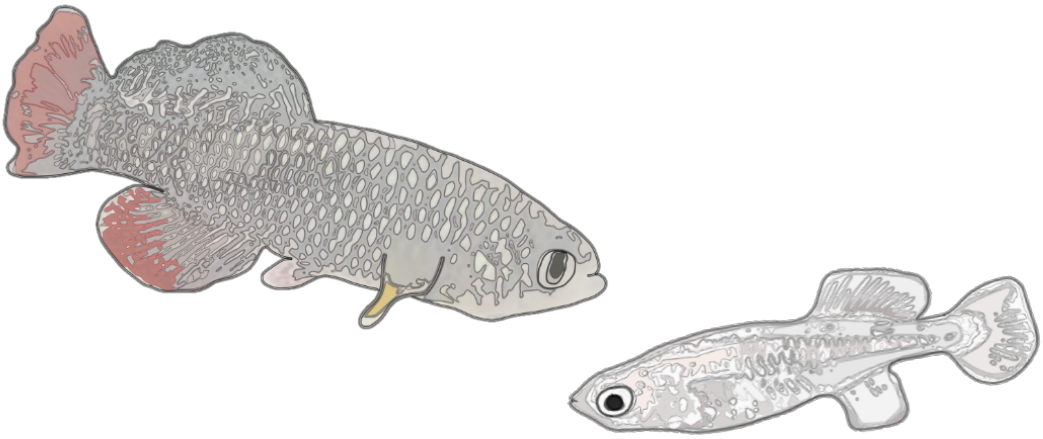
Social behaviour of the African turquoise killifish (*Nothobranchius furzeri*)



Submitted by Caitlin Searle to the University of Exeter as a dissertation for the degree of Masters by Research in Psychology January 2019

I certify that all the material in this dissertation which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other university.

Abstract

To date, very little is known about the African turquoise killifish and its social behaviour. It is emerging as a model organism in gerontological research due to its exceptionally short lifespan (approximately 6-8 months), yet its social behaviour is unknown. In this study, I investigate the grouping tendencies of juveniles using different methodologies to determine which best suits the species. Secondly, using the most suitable methodology, I investigate the grouping preferences of both juveniles and adults and observe whether they are capable of familiar and kin recognition. To my knowledge, this is the first study to investigate the grouping preferences and recognition capabilities of the African turquoise killifish. Using 7-week-old juveniles I compared a standard binary choice and Y-maze test arena and determined that the Y-maze was not only a more suitable test arena for my study species, but that at this age juveniles grouped. Using the Y-maze for further testing I investigated differences in juvenile and adult behaviour. I observed that at 5 weeks old juveniles displayed no preference for grouping, kin or familiar individuals. In contrast, adult killifish showed a preference for grouping and males demonstrated a preference for familiar male individuals. Similar to juveniles, adult killifish showed no preference for kin, nor did females show any preference for other familiar females. This work provides a framework for future studies to investigate this species further and increase our knowledge on both the African turquoise killifish and, more generally, on ontogenetic shifts in social behaviour.

Acknowledgements

Firstly, I would like to thank my supervisors, Darren and Tim. Without their support and guidance, I would not have been able to complete this Masters. Thank you, Darren, for introducing me to these wonderful fish and for your patience and explanation on everything social behaviour related and for buying me food I could eat during our breakfast meetings. Tim, though R and I will never be the best of buddies, we are now good acquaintances because of you. At one point, I even remember saying how much I was enjoying model averaging.

A huge thank you must go to the people in the fish laboratory; Chloe, Sylvia, Christine, Adam and even Rob. You were immensely helpful in designing the project and were there for all the great times and all the catastrophes.

I have to thank the people in the Crab lab. You made working on fish that were always dying, bearable. From going to the pub to winning squash, I always had a smile on my face when working with you guys.

To my family and friends, some of whom literally had no clue what I did, thanks for being there to rant to and read through drafts and ask me about my job prospects after this, without you guys, I couldn't have done it.

Finally, thanks to Pasty for endless cuddles and reassurance that animals are the best thing to work with.

Table of Contents

List of Figures

Chapter 1

Chapter 2

Chapter 3

List of Tables

Chapter 3

Chapter 1: General Introduction

1.1 Group living

The formation of social groups is one of the most prominent and wide-spread aspects of animal behaviour (Krause & Ruxton, 2002). It is common in numerous species and is influenced by the characteristics and composition of the individuals living within the groups and the interactions between these individuals (Mills & Marchant-Forde, 2010). A sociality spectrum exists in terms of how regularly these interactions take place and how complex they are. At one end, we see relatively simplistic behaviour between one or more individuals that temporarily interact to access certain resources. Examples of this include groups of wildebeest (genus: *Connochaetes*), zebra (genus: *Equus*), elephants (family: *Elephantidae*) and buffaloes (genus: *Syncerus*) that form temporary feeding aggregations at watering holes (Gereta et al., 2004), fish that form aggregations at cleaning stations to gain services from cleaner wrasse (*Labroides dimidiatus*) (Slobodkin & Fishelson, 1974) and sage grouse (*Centerocercus urophasianus*) that form leks at communal display grounds to attract females (Wiley, 1978). At the opposite end of the spectrum we see complex and dynamic group living in which societies share resources, co-operate and divide labour. The most extreme form of this is represented by eusociality in insects and naked mole rats (*Heterocephalus glaber*) (Jarvis, 1981; Wilson & Hölldobler, 2005). Additionally, at this end of the spectrum, we see individuals that are capable of caring altruistically for young and forming dominance hierarchies. The former apparent in co-operatively breeding birds such as the Florida scrub jay (*Aphelocoma coerulescens*) (König, 1997), and the latter, common in primate societies (Bernstein, 1976).

The patterns of social behaviour expressed by a population are a consequence of the social stimuli and the physical environment encountered by an individual and their grouping preferences (Mills & Marchant-Forde, 2010). A preference for grouping should only occur if there is a net fitness benefit to being a part of a group, otherwise an animal should remain solitary. Many fish species are known to spend a significant amount of time in social groups (Pitcher, 1989). These are highly dynamic, with individuals joining and leaving groups that vary in size and composition in response to both the environment and their own internal state (Hoare et al., 2000). Groups are often formed for foraging, spawning or anti-predator reasons and as a result of a trade-off between the costs and benefits of these factors.

1.1.1 Benefits of group living

From the perspective of prey, group membership provides a multitude of benefits through anti-predator defences (see Krause & Ruxton, 2002 for a review). Despite a larger group having a greater probability of being detected, if a predator can only consume a certain amount of prey in a single attack, group living decreases the chance of any one individual being predated upon and gives rise to the dilution effect (Foster & Treherne, 1981). For example, sea skaters (*Halobates robustus*) decrease their chances of being caught by fish predators by forming large groups (Foster & Treherne, 1981), stenogastrine wasps (*Liostenogaster vechti* and *L. flavolineata*) nest in large clusters to avoid their invertebrate predators (Coster-Longman et al., 2002) and sand fiddler crabs (*Uca pugilator*) form more cohesive groups not only due to higher predation threats but also for risk dilution (Viscido & Wethey, 2002). Further examples in fish include banded killifish (*Fundulus diaphanous*), which choose to associate with fish in a

larger group when they are presented with a choice between two different group sizes after a predation event (Krause & Godin, 1994; Krause & Ruxton, 2002), Trinidadian guppies (*Poecilia reticulata*), which form tighter and more cohesive groups when experiencing high predation events (Huizinga et al., 2009), and European minnows (*Phoxinus phoxinus*), which are known to form very cohesive groups in the presence of predators (Magurran, 1990).

The probability of survival is increased if an individual is able to detect and avoid the predator. When living in a group there are more animals scanning for predators. This not only increases the chances of the predator being detected but also allows an individual to devote more time to foraging without increasing its predation risk. This premise was first proposed by Pulliam (1973), who noted that finches in flocks would trade off foraging with vigilance bouts to actively scan for predators and as group size increased the likelihood of a predator being spotted increased (many eyes hypothesis). This has been demonstrated in multiple fish species including cyprinids (goldfish (*Carassius auratus*) and minnows), in which larger groups spent more time feeding and found food faster as they were less timid and had more access to information from others (Pitcher et al., 1982; Pitcher et al., 1983; Pitcher, 1986), and ocean surgeonfish (*Acanthurus bahianus*) that were able to forage for longer periods of time in schools than they could if they remained solitary (Wolf, 1987).

This vigilance is not only confined to prey groups: top carnivores such as the spotted hyena (*Crocuta crocuta*) have been found to show increased vigilance when detecting interspecific threats (Pangle & Holekamp, 2010). Through quicker

detection of the threat, individuals can spend more time on other activities that increase fitness. In addition to the benefits provided through increased vigilance and avoidance, group living may benefit prey through the confusion of predators. As group size increases, a predator can suffer sensory confusion as prey are closely grouped and are physiologically similar (Landeau & Terborgh, 1986). Predators can experience difficulty attacking a single individual as there are many stimuli at one time, hence giving rise to the confusion effect (Shaw, 1978). Previous work, such as (Hebblewhite & Pletscher, 2002), has suggested that as group size increases the likelihood of a predator spotting a group can also increase. Contrary to this, more recent work using 3-spined sticklebacks as a predatory species, suggests that in fact the encounter rate decreases as prey aggregations become larger as there become fewer prey groups and fewer opportunities for predation (Ioannou et al., 2011).

Another potential benefit of group membership is that individuals can gain knowledge on the location of food, the ability to acquire the food and a mutual interest in defending the food from others. Ward and Zahavi (1973) discuss the use of 'information centres' in relation to bird colonies and suggest that groups of individuals have evolved for the exploitation of food sources, by the sharing of information. Examples of vertebrates that demonstrate this type of information sharing include ravens (*Corvus corax*), who learn the location of food through others in their roost (Wright et al., 2003), red-billed diochs (*Quelea quelea*), where eavesdropping individuals learn the location of food from observing successful foragers within their group (Ward, 1965), and naked mole rats, who communicate when foraging and give special vocalisations to indicate that a food source has been found (Judd & Sherman, 1996). However, once a food source has been

located, it must be acquired, and this acquisition can require co-ordinated behaviour between individuals. In bottlenose dolphin (*Tursiops truncates*) societies, individuals have distinctive behavioural roles which increase the chance of prey capture success (Gazda et al., 2005). One individual will be the 'driver' and push the food source to other individuals known as 'barriers', who prevent it from escaping. This collective behaviour increases the chance of a successful hunting event and all individuals benefit from increased food intake.

Group membership can also offer benefits in terms of defence of a resource. Where food is patchily distributed, it is often likely that many individuals will be competing for the same resource. When this occurs it is difficult for an individual to defend this resource alone. Hence, being part of a stronger, larger group increases food acquisition possibilities. In lions (*Panthera leo*), for example, when a group contains a male and is larger, it is more likely to be able to defend food against hyenas, whilst smaller, weaker, all-female groups would lose out on the food source (Cooper, 1991).

Group living can also influence reproductive success, as an individual is more likely to find a mate in a group environment due to the high density of individuals in one area. Breeding success is similarly increased if a greater number of individuals are looking after young (cooperative breeding). This is known to confer multiple benefits to both young and adults, allowing adults to spend more time foraging, whilst young individuals are protected from predators by multiple older individuals. Seychelles warblers (*Acrocephalus sechellensis*), amongst other birds, cooperatively breed and care for young, which not only increases their own

chance of reproduction, but increases the survival chance of both their young and the young of other group members (Komdeur, 1992).

1.1.3 Costs of grouping

As group size increases the costs associated with group living often also increase. Increased group size can increase foraging competition. Molvar and Bowyer (1994) found that despite Alaskan moose (*Alces alces gigas*) foraging time increasing with increased group size, their foraging efficiency decreased due to an overriding effect of intra-group aggression. Similarly, in goldfish, as group size increases, competition for food subsequently increases and fish have to decrease food handling time (Pitcher, 1989). Costs may not be equal for all individuals, particularly if they adopt a hierarchical structure, meaning not all group members are equal in rank. Alados and Escós (1992) found that in hierarchical groups, lower-ranking Cuvier's gazelles (*Gazella cuvieri*) suffered reduced fecundity, reduced offspring survival and increased age at first birth compared to higher-ranking individuals. Despite these great costs, lone gazelles risked increased predation. Therefore, solitary life and group living represent different resolutions of the trade-off between survival and reproduction.

Though group living may increase the chance of finding a mate and improve offspring survival, it can also have negative effects on breeding. Groups that are isolated from others risk reduced gene flow and genetic variation, which can result in inbreeding. This can have serious negative consequences for individuals such as a higher number of deformed offspring, a greater risk of stillborns and individuals becoming sterile. This has been demonstrated in adders (*Vipera*

berus), where an isolated population suffered these issues until males from another population were introduced manually (Madsen et al., 1996).

Many animals are susceptible to pathogens and parasites and in a group situation, it is possible that transmission of these is increased (Krause & Godin, 1996). For example, when observing colonies of cliff swallows (*Petrochelidon pyrrhonota*), larger colonies had a greater level of transmission of parasitic bugs than smaller colonies (Brown & Brown, 2004). Whilst in guppies, parasite transmission and infection contraction is higher in females, as females have a greater propensity to group (Johnson et al., 2011). Transmission of disease in groups is not uncommon and may be further exacerbated if competition for food and resources is apparent (Côté & Poulinb, 1995). Some individuals may possess physical injuries or be under high-stress situations, thus increasing this transmission risk further as they are more susceptible to infection. Despite disease risk being a major cost of group living it could also provide an incentive for animals to live in groups, as grooming of others removes parasites and pathogens more efficiently and therefore provides another benefit of sociality (Pérez Pérez & Veà, 2000).

In summary, group membership is important for many animals for access to certain resources. Individuals acquire greater access to information and associated benefits, mating opportunities and protection against predation than they would if they remained solitary. Despite this, grouping can confer costs. Disease transmission, inbreeding depression, aggression amongst group members and increased visibility to predators are amongst the costs individuals

in groups are at risk of. The costs and benefits an individual is subjected to dictates whether group living is more beneficial than solitary living.

1.2 Variations in the level of recognition

It is likely that if an individual is a member of a group, they have the ability to recognise others, even to the most basic degree (Krause & Ruxton, 2002). This is due to the fact these animals are interacting repeatedly over time and there is a necessity for the differential treatment of individuals based on a variety of social contexts. Variation in recognition is explained by the costs and benefits associated with the necessity to recognise conspecifics, the mechanisms behind this recognition and the cognitive ability of the individual in question (Tibbetts & Dale, 2007).

1.2.1 Basic recognition

A common example of basic recognition would be that used in large groups of animals, such as shoals, herds or swarms, where not every individual will know every other member of the group but will recognise the fact they are similar in their phenotypes or actions. For instance, in social wasps (genus: *Polistes*), individuals discriminate between nest mates and other conspecifics, but may not recognise specific individuals due to the high density of wasps within the nest (Gamboa et al., 1986). Most animal groups contain 1 species as assorting in this manner means that individuals of similar size, body length, colour and species are in the same group (see Shaw, 1978; Krause et al., 2007 for reviews), reducing the chance of predation via the oddity effect (Landeau & Terborgh, 1986). However, some animals form groups that contain 2 or more species. These

groups can occur in many habitats and vary dependent on the season and the presence of predators. These mixed species groups confer two main advantages; a foraging advantage and predator avoidance and have been observed in many types of bird and coral reef fish (Stensland et al., 2003). Examples of fish that assort via phenotypic traits include banded killifish, which have been found to associate preferentially with individuals of a similar size, even if the individual is of a different species (golden shiners, *Notemigonus crysoleucas*; (Krause & Godin, 1994; Krause & Ruxton, 2002), western rainbow fish (*Melonotaenia australis*), which, under laboratory-induced colouration changes, choose to associate with those that have a similar colour pattern to their own (Rodgers et al., 2010), and mollies (*Poecilia latipinna*), reared in isolation, which choose to group with fish of the same colouration (Ledesma & McRobert, 2008).

1.2.2 Familiarity and kin recognition

In stable social groups where individuals repeatedly interact, it may be possible for an animal to be able to recognise certain individuals and preferentially associate with them. Many fish species are capable of a more sophisticated level of recognition and can distinguish between related, familiar and specific individuals and group accordingly. For example, fry of three-spined sticklebacks spent more time associating with kin when predators were present (Fitzgerald & Morrissette, 1992). Male blue gill sunfish (*Lepomis macrochirus*) are capable of recognising offspring over non-kin fry through self-referent phenotype matching, biasing their care towards their own (Neff & Sherman, 2003), whilst groups of familiar fathead minnows are able to detect the chemical stimuli of predators and group more cohesively (Chivers et al., 1995).

The learned recognition of individuals that are not necessarily related to one another can be split into two main types. Context-dependent recognition refers to the recognition of individuals based on previous encounters in association with specific cues and can occur over a very short timeframe (Griffiths, 2003). This type of recognition occurs in situations where the costs and benefits are great. Examples of this include predator inspection and foraging (Griffiths, 2003). In sticklebacks that have only associated for a short amount of time (less than a day), the same pairs of individuals repeatedly performed predatory inspections together (Milinski et al., 1990). Individuals in this study were found to associate with the same partner significantly more than expected by chance, suggesting in this context, they recognise their preferred partner (Milinski et al., 1990). Similarly, in guppies, naïve fish chose to associate with experienced and successful foragers that informed the fish to the location of food patches, increasing their foraging success (Lachlan et al., 1998).

Contrastingly, context-independent recognition, where individuals are recognised based solely on previous interactions and in the absence of any obvious morphological or behavioural characteristics, is generally termed recognition of familiars (Griffiths, 2003). Via this mechanism, individuals that have been in regular contact can recognise each other and closely associate. Examples of this include humboldt penguins (*Spheniscus humboldti*), which are capable of discriminating between familiar and unfamiliar non-kin odours, which means they are able to locate colony mates and burrows at night (Coffin et al., 2011), guppies, which prefer to shoal with familiar over unfamiliar individuals regardless of kinship (Griffiths & Magurran, 1999), and three-spined sticklebacks (*Gasterosteus*

aculeatus), which can recognise familiar unrelated potential mates and reduce their aggression towards them (Utne-Palm & Hart 2003).

Finally, individual recognition is the most complex form of recognition and refers to the ability of an individual to be able to recognise another using their distinctive characteristics, with no prior association (Tibbetts & Dale, 2007). It is usually associated with another form of recognition, such as kin recognition and depending on the context, can be used to discriminate between mates, siblings or rival individuals (Tibbetts & Dale, 2007). This type of recognition is common between parent and offspring and can occur when animals live in large groups and must find specific family members. Mexican free-tailed bat mothers (*Tadarida brasiliensis mexicana*), recognise their pup's vocalisations, leading to successful mother-pup reunions after time apart (Balcombe, 1990), whilst royal terns (*Sterna maxima maxima*) are capable of recognising their eggs based on their colouration and superficial markings (Buckley & Buckley, 1972).

Recognising kin, familiars and specific individuals provides greater benefits than basic recognition, such as the ability to care altruistically for young, avoid inbreeding and strengthen anti-predator defences. Despite this, if sophisticated recognition is not required, individuals can benefit from simply discriminating on the basis of species identity (conspecifics versus heterospecifics) or phenotypic differences.

1.3 Recognition mechanisms

As individuals differ in their ability to recognise conspecifics, so do their mechanisms behind this recognition. Sherman (1997) considers three main components to be important when recognising other individuals: production, perception and action. These components refer to an interaction between two or more individuals. For example, an animal (sender) produces a cue which is received by another animal (receiver), the receiver then analyses this cue using its own recognition template (identification of others through representation of certain key characteristics) (Mateo, 2004). Depending on the type of cue, its quality and the sophistication of the receiver's recognition template, the animal receiving the cue may be able to deduce the sender's identity at the very least, to conspecific or heterospecific level (Sherman et al., 1997). Once they have identified the sender, the receiver can then adjust their actions and behave accordingly. The sensory cues used to communicate vary and may be of acoustic, visual or chemical form. Cue use depends on habitat and social organisation preferences and individuals are not confined to using one method; a combination provides receivers with a multitude of cues they can reference against their own, increasing the likelihood of recognition (Rybak et al., 2002).

1.3.1 Visual cues

Visual cues such as variation in size, colouration, species and sex are used by individuals so they can join a group in which members are phenotypically similar (Ward & Hart, 2003), thus benefitting from the aforementioned decrease in the oddity effect and increased spatial proximity (Landeau & Terborgh, 1986; Krause et al., 2000). Examples of this type of cue use include paper wasps (*Polistes fuscatus*) that show variability in the yellow markings on their face and abdomen

and use these to individually recognise and interact with each other (Tibbetts, 2002), male blackcaps (*Sylvia atricapilla)* that can associate species-specific plumage with species-specific song and retain this memory for a long period of time (Matyjasiak, 2005) and zebrafish (*Danio rerio*), which when reared with individuals with particular colour patterns, regardless of whether they were similar to their own, preferred to group with these individuals (Spence & Smith, 2007).

1.3.2 Acoustic cues

The use of sound to communicate is very common in the animal kingdom and animal vocalisations are used by a great range of taxa (Cheng et al., 2010). High levels of specificity can be gained through this type of cue use. Ewes of domestic sheep (*Ovis aries)* can recognise and preferentially care for their lambs through their calls (Searby & Jouventin, 2003) and king penguin chicks (*Aptenodytes patagonicus)* can recognise the calls of their parents over the continuous background noise of a colony (Aubin & Jouventin, 1998).

1.3.3 Chemical cues

In some species, the production of pheromones and odours may elicit the recognition of other individuals. Whilst the cue is chemical, the mechanism by which it is received is olfactory. Unlike visual and acoustic cues, chemical cues can persist (to an extent) over time and can be identified by others even if the sender of the signal is no longer in the area (Ward & Webster, 2016). Wolves use scent-marking (urination, defaecation and scratching) to establish and maintain pair-bonding (Rothman & Mech, 1979), zebra finch fledglings (*Taeniopygia guttata)*, use chemical cues to distinguish between kin and non-kin individuals

and prefer the nest odour of close relatives (Krause et al., 2012), and mice (genus: *Mus*) discriminate not only between male mice but between two different species using the odours they produce (Bowers & Alexander, 1967). Commonly associated with chemical cues and olfaction, is the major histocompatibility complex (MHC). The MHC is a large chromosomal region containing closely-associated, highly polymorphic genes that play an important role in non-self-recognition (Penn & Potts, 1999). Its usage has been demonstrated in a number of species including mice, in which house mice preferred mates that had different MHC's to themselves (Penn & Potts, 1999), in fish, in which sticklebacks used odour-based selection to determine whether potential mates had optimal levels of MHC diversity for their offspring (Milinski et al., 2005) and in humans, where females preferentially associated with the odours of males with dissimilar MHC's to their own (Wedekind et al., 1995).

1.4 Ontogenetic effects on group living

Few organisms show consistent patterns of social organisation throughout their lives. Changes to grouping preferences reflect the dynamic environment in which individuals live and their life-history adaptations. Whilst grouping may be beneficial at some points during an individual's life, at others it can be detrimental and costly. The balance between these benefits and costs dictates whether grouping is favoured at each life stage. Hence, we see such a range of grouping preferences both within and between species. Some animals group early in life, whilst others do not develop a grouping tendency until later in life. These ontogenetic shifts in behaviour are organism-dependent.

1.4.1 Grouping as juveniles

The grouping tendencies of juveniles and adults are likely to vary due to the different selection pressures they face. For juveniles, grouping confers benefits such as anti-predator defence and the opportunity to socially learn, which assists in future interactions. The incentive to group may decrease as individuals become older as they may experience less predation events and the cost of intraspecific competition for food and mates may increase. This early-life sociality, where juveniles are more social than adults, is common in numerous taxa from terrestrial to marine animals. For example, despite male African elephants being social throughout their entire lives, their level of sociality varies during their lifespan. Adolescents prefer to be in larger social groups than their older male counterparts as during this time there is an intense period of learning and development (Evans & Harris, 2008). Several species of squamate reptiles (order Squamata) remain in their natal group until they are ready to disperse as adults, suggesting a benefit to remaining together while young (Chapple, 2003). Juvenile eels (order Anguilliformes), exhibit schooling behaviour during the migratory phases of their lives, yet as adults they act antagonistically towards one another, resulting in severe injuries and cannibalism (Edeline et al., 2009). Whilst lemon sharks (*Negaprion brevirostris*) group for protection as juveniles and are predominantly solitary as adults (Guttridge et al., 2009).

1.4.2 Grouping as adults

Ontogenetic changes in motivation and social structure result from both maturation, which involves intrinsic processes, and experience with the environment (Pitcher, 1989). Animals that begin grouping later in life do so for similar reasons to juveniles: for anti-predator advantages and access to

resources. Their lack of grouping at a juvenile stage could be explained by the added factor of mate choice and the reproductive advantage of living in a group. Caribbean spiny lobsters (*Panurilus argus*) show large ontogenetic shifts in their social behaviour throughout their complex life history and whilst adults are highly social and benefit from group membership through co-operative group defence, juveniles are solitary (Dolan & Butler, 2006).

1.4.4 Limitations in ontogenetic research on fish

Ontogenetic shifts in sociality are common in many fish species yet are rarely studied within a fish's lifespan. There are few studies addressing this shift in sociality as in laboratory research the most commonly used fish include guppies, zebrafish, sticklebacks and fathead minnows (Polacik et al., 2016). Unlike non-vertebrate model organisms, in which the majority of lifespans are relatively short, these fish can live for longer periods of time. This long lifespan means that ontogenetic shifts in behaviour are not usually quantified due to research constraints, such as the repeatability of results and the feasibility of multiple experiments over numerous years (Hu & Brunet, 2018). In the wild, studies encounter different barriers. There are difficulties in capturing entire shoals, determining their predation pressures and foraging capabilities and observing these for an entire lifespan. It is possible, yet requires exhaustive monitoring of populations for multiple years. An example of a type of study that addressed this ontogenetic shift is a study on over 500,000 pacific herring (*Clupea pallasii*), whose grouping behaviour was observed for 14 years. In this study, herring were shown to maintain groups over several years and long distances (Hay & McKinnell, 2002). Despite the novelty of this study it provides valuable information on the ontogeny of sociality. However, more studies are required to provide a

reliable insight into the changes in grouping behaviour over a lifespan. Before now research constraints on laboratory species have limited the possibility of these studies. Addressing this shortfall requires a vertebrate model organism that is intrinsically short-lived. A species that could enable this type of research to occur is the African turquoise killifish. The African turquoise killifish is a species that is both intrinsically short-lived and has been successfully utilised in other aspects of scientific research.

1.5 African turquoise killifish

Killifish are egg-laying fish within the cyprinodont clade (order Cyprinodontiformes) and include species with both annual and non-annual lifespans (Polacik et al., 2016). Annual killifish inhabit temporary pools that form during the monsoon season in South America and Africa and live on average for less than a year (Polacik et al., 2016). This adaptation is due to their habitat desiccating during the dry season and means they must undergo sexual maturation and maximise reproduction within this short timeframe.

Among annual killifish species, the African turquoise killifish (*Nothobranchius furzeri*) is the shortest-lived (Jubb, 1971), and in fact is the shortest-lived vertebrate to be bred in captivity, with a median lifespan of approximately 6 months and a maximum lifespan of 9-10 months (pers.obs - CS 2017). This short lifespan is intrinsic and not an artefact of laboratory conditions (Vrtílek, Žák, Polačik, et al., 2018). The , African turquoise killifish have evolved this life history adaptation in response to the ephemeral nature of the pools they inhabit (Polacik et al., 2016).

1.5.1 Habitat and physiology

African turquoise killifish are an ephemeral pool-dwelling species with a widespread geographical distribution throughout tropical areas of Eastern Africa (Cellerino et al., 2015; Froese & Pauly, 2017). Their habitat is both temporary and unpredictable, therefore juveniles rapidly sexually mature (at approximately 7-8 weeks old dependent on diet and strain; (Polacik et al., 2016)) and, once sexually mature, produce eggs on a daily basis. These eggs are desiccation-resistant and remain in a state of developmental diapause during the dry season, hatching once triggered by extrinsic factors when the ephemeral pools return (Cellerino et al., 2015). Adults show marked sexual dichromatism and dimorphism: males have bright colouration and a larger body size than females (Fig 1). Males occur in two main colour morphs (red and yellow), and this colouration is primarily observed on the pectoral and caudal fins. Females are translucent in colour and smaller than their male counterparts.

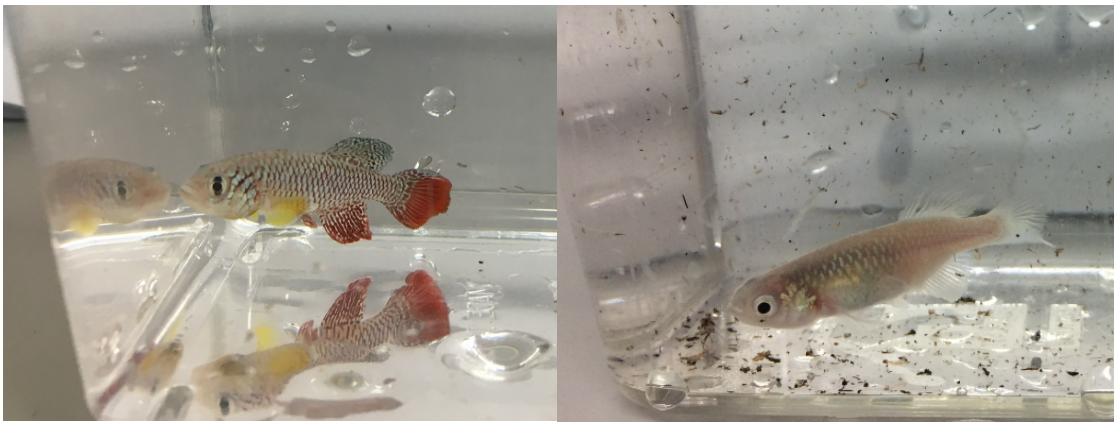


Figure 1: ***left*** Adult male killifish, red morph, ***right*** Adult female killifish

1.5.2 Use as a model organism

The majority of the literature on the lifecycle of African turquoise killifish has arisen from their increasing popularity in gerontological research using captive populations (Cellerino et al., 2015; Harel et al., 2016). Their aging can be modified and manipulated by changes to water temperature (Valenzano Dario et al., 2006), diet (Terzibasi et al., 2009; Cellerino et al., 2015) and polyphenol addition (Terzibasi et al., 2007). Their short generation time provides opportunities for powerful genetic analyses of age-related diseases and vertebrate aging (Harel et al., 2016).

1.5.3 Future use

As stated above, due to their compressed lifespan, African turquoise killifish are being used as a model organism at the forefront of gerontological research. Yet this lifespan length means that African turquoise killifish can be used to ask fundamental questions about behavioural ontogeny from juveniles to adults. Currently however very little is known about the within-lifespan changes to the sociability of fish species, including changes to their grouping preferences across development. The benefits and costs of grouping are not likely to be consistent throughout an individual's lifespan therefore it is similarly unlikely that their grouping and social tendencies will be unvarying. The African turquoise killifish's lifespan length enables research to be conducted in a short period of time, as an entire population's social preferences can be analysed in less than a year. Increased knowledge on their behavioural tendencies will further both our understanding of the behavioural ontogeny of multiple fish species and our understanding of the African turquoise killifish's short, yet complex life history.

1.6 Thesis outline

The aim of this chapter was to demonstrate the diversity of group-living behaviour across the animal kingdom and how this behaviour is affected by both internal and external factors. I have discussed the benefits and costs of group membership and how variations in these benefits and costs determine whether individuals choose to join or leave groups, giving rise to the range of dynamics we observe.

I have also discussed how grouping behaviour varies depending on differences in the type and sophistication of recognition mechanisms. If a greater level of recognition is present, animals may be capable of recognising kin or familiar individuals, with some able to recognise specific individuals. Recognition cues may be based on an unlearnt template or learned as an individual ages, in which case ontogenetic shifts in recognition occur and give rise to changes in behaviour.

Grouping preferences have been studied in many fish, but due to their life-history adaptations and long lifespans, the majority of studies have not investigated ontogenetic shifts in behaviour. Thanks to its rapid development and short lifespan, the African turquoise killifish provides a suitable model system to study how grouping changes as an individual ages. The present study aims to find out whether African turquoise killifish are social and have a preference to group at both juvenile and adult stages, and whether this is biased towards related or familiar individuals. This work provides the first experiments on African turquoise killifish behaviour and aims to increase the understanding of this fish's social

preferences. In **Chapter 2** I examine whether African turquoise killifish have a preference to group. Specifically, I ask (1) whether grouping is apparent at different stages during juvenile development and (2) what is the best experimental set-up for investigating social preferences in African turquoise killifish, in which nothing is known about their association preferences. Having established that African turquoise killifish are social and developed an appropriate method to quantify social preferences in the species in **Chapter 3** I go on to investigate (1) whether juvenile and adult African turquoise killifish differ in their preferences for grouping and (2) whether both are capable of recognising kin and familiar individuals. Finally in **Chapter 4,** I provide a general discussion for the thesis and synthesis the main findings in chapters 2 and 3 in the context of the wider literature.

Chapter 2: Do juvenile African turquoise killifish show a grouping preference and is the current methodology a suitable test for this?

2.1 Introduction

Many marine and freshwater fish are social, with the majority of these species spending a large proportion of their lives in groups. Groups can vary in their cohesiveness and structure and are influenced by the selection pressures arising from environmental and social conditions, acting on the individuals. Some fish, including zebrafish (*Danio rerio*) (Engeszer et al., 2007), guppies (*Poecilia reticulata*) (Lindström & Ranta, 1993) and fathead minnows (*Pimephales promelas*) (Stumbo et al., 2012), form loosely cohesive shoals that engage in social behaviours and reap the benefits of grouping, but are not highly organised. Others, including Atlantic herring (*Clupea harengus*) (Pitcher et al., 1985), barracuda (genus: *Sphyraena*) (Partridge, 1982) and Atlantic mackerel (*Scomber scombrus*) (Pitcher et al., 1985) exhibit remarkably co-ordinated movements and form tightly structured schools. Both types of groups (shoals and schools) can confer foraging benefits (Pitcher et al., 1982), increased mating capabilities (Ruhl & McRobert, 2005) and better anti-predator defences (Major, 1978).

Laboratory experiments have deepened our understanding of grouping in fish and how it varies depending on the selection pressures acting on the individuals. The standard binary choice test is one of the most commonly used methods when investigating behavioural choices and previous work has demonstrated its flexibility in exploring different aspects of social preference (Fig 1). Its usage ranges from small- to large-scale experiments and it has been used to investigate information transfer of patch size in fifteen-spine sticklebacks (*Spinachia*

spinachia) (Webster & Laland, 2017). Similarly, it has been used to investigate size-assortative shoaling driven by hierarchy of individuals in juvenile angelfish (*Pterophyllum scalare*) (Gómez-Laplaza, 2006), and size-assortative grouping due to predation in guppies (Jones et al., 2010). Finally, it has been used to investigate preferences for size-matched conspecifics in lemon sharks (*Negaprion brevirostris)* (Guttridge et al., 2009).

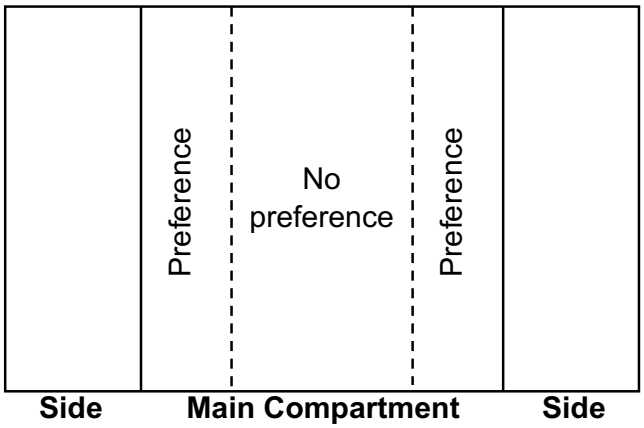


Figure 1: 2D diagram of the standard binary choice test arena. The individual being tested is contained within the main compartment, whilst stimuli to the test individual are housed in the side compartments. The time the test individual spends within the 'preference' and 'no preference' zones (highlighted by the dashed lines) is then measured. Preference for the stimulus in one side compartment is inferred when the individual spends significantly more time in that preference zone than the other.

When investigating preference for different groups, generally both compartments contain stimuli and the focal fish must decide which to join, based on both internal

and external factors. For example, using this method, Binoy et al. (2015) found that climbing perch (*Anabas testudineus*) used visual cues when exhibiting a preference for familiar conspecifics, whilst Morrell et al. (2007) found that guppies preferred to group with those that had recently experienced a similar diet to themselves.

At its most basic level, when testing grouping tendencies, one side compartment contains stimulus fish whilst the other remains empty. This set-up allows the investigator to observe whether the focal fish has a preference to be social (i.e. to associate with others) or remain solitary. This method has been used for a range of fish species to understand whether they are social (Snekser et al., 2006; Engeszer et al., 2007), and is useful when looking at a fish species in which the grouping behaviour was previously unknown.

In the present study, I examine the grouping preference of the African turquoise killifish (*Nothobranchius furzeri*), a teleost fish. African turquoise killifish inhabit ephemeral pools in Mozambique and Zimbabwe, in which they hatch, rapidly sexually mature (7-8 weeks) and live for approximately 6-8 months due to desiccation of their habitat (Polacik et al., 2016). In contrast to other vertebrate model organisms, their short lifespan provides opportunities for research that were previously unavailable.

Due to their cryptic habitat preferences and annual lifecycle, very little is known about the behaviour of African turquoise killifish. In particular it is currently unknown if the species is social and demonstrates a preference to associate with

conspecifics. In this study, I determine whether African turquoise killifish have a preference for associating using a standard binary choice test. I measure the preference to group at 3 and 5 weeks old, when juvenile, and at 7 weeks old, when the killifish are thought to be beginning their sexual maturation but sex cannot be determined by eye.

2.2 General Methods

2.2.1 Breeding Individuals

F1 breeding individuals were housed in aquaria (80 (length (L)) x 40 (width (W)) x 30 (depth (D)) cm) in a large group (~25 individuals), and were fed a mixed diet of brine shrimp nauplii (*Artemia salina)* and bloodworm (*Glycera dibranchiata*). Fish were commercially available (population origin unknown), purchased from Aquasense Ltd (Bucks, UK). Small spawning tanks (18L x 11W x 11D cm) were placed inside the aquaria and checked daily for eggs. Once collected, eggs were transferred to transparent petri dishes (large: 9 cm, small: 5.5 cm circumference) and underwent methylene blue treatment for 24 hours to minimise fungal infection, as suggested by published protocols (Polacik et al., 2016). They were then kept in moist peat and checked daily for approximately 3 weeks, until 'golden eyes' were observed and they were considered ready for hatching (Polacik et al., 2016).

2.2.2 Experimental Subjects

From hatching, juvenile fish were kept in 10 groups of 4 individuals in transparent aquaria (22L x 13W x 13D cm) that were visually isolated from other group tanks. Temperature was maintained at 24.31 °C ± 0.12 (mean ± standard deviation

(SD)) and illumination was set on a 12:12 light:dark cycle. Fish were fed uniform drops of brine shrimp nauplii daily. To control for any feeding order effects before experimentation, feeding order across tanks was randomised. Water changes were carried out at 2-week intervals.

This experiment used the individuals (1.83 cm ± 0.31) from the 10 groups, each tested once. Unfamiliar stimulus fish (1.87 cm ± 0.37, n = 23) from the same generation were chosen randomly from a separate stock tank (40L x 23W x 29D cm). Any test fish that died were replaced, where possible, with fish kept under the same conditions; where this was not possible the remaining fish were left in their group and no further fish were added. Focal fish were not used as stimulus fish and vice versa. Upon hatching and again at the time of testing, groups were transferred to a large square petri dish (12L x 12W x 1.5D cm) and size of both the focal and stimulus fish were measured using photographs taken from above with an iPhone 5S. Measurements were taken of each individual's length from the jaw to the caudal peduncle using ImageJ photo analysis software (Rasband, 2017). The images were spatially calibrated from pixels to cm using a 30cm ruler which was placed in every photograph. A distance of 1cm was measured and set as a known distance for each picture therefore when measuring, the distance was converted from pixels to cm.

2.2.3 Ethical Note

This research was approved by the University of Exeter Psychology Ethics Committee (ref. 2017/1658). Fish used were bred from the F1 generation of laboratory fish, meaning no transport of experimental fish was necessary.

Behavioural tests did not involve any physically invasive manipulation. After the experiment, all fish were returned to standard housing tanks.

2.3 Experiment 1 – Standard Binary Choice Tests

In this experiment 39 individuals were tested at 3, 5 and 7 weeks old to see if propensity to group changed with age. The experimental tank was a glass aquarium, filled with 6 cm water at 23.4 °C ± 0.14, with three compartments separated by transparent perforated Perspex (Fig 2). This allowed both visual and olfactory contact between the focal and stimulus fish. Using Pitcher's (1983) elective group size (EGS) criterion I measured social interactions as focal fish being within 4 body lengths of the stimulus group, creating zones that were approximately 4 body lengths distance from the stimulus group. As the EGS of African turquoise killifish is unknown and this being the most commonly used method for measuring social interactions, this criterion was used. The main compartment consisted of three marked zones, a 'Social', 'Non-social' and 'Neutral' zone, and housed the focal fish during the experiment. Each focal fish was given a 2-minute settling period in the main section. Pilot experiments showed that within this time the majority of fish had explored all areas of the compartment and resumed their normal behaviour (pers. obs. CS 2017).

One of the two side compartments contained three fish from the stimulus group, which had been added at the same time as the focal fish, whilst the other remained empty. During the two-minute acclimation time stimulus fish were in both visual and olfactory isolation from focal fish. Once the barrier was removed the focal fish were observed for ten minutes and the time spent in each zone was

recorded. This measurement was collected via a camera set up above the tank, whilst the tank was hidden behind a curtain, thus avoiding disruption from the experimenter and other external stimuli. Trials were video-recorded using a Panasonic HC-V750 camcorder. The side of the stimulus group was alternated between trials to control for a lateral side bias. Water in the tank was changed between each focal fish.

Preference was measured as the proportion of time spent being social as opposed to being solitary, calculated as:

$$\frac{\textbf{Time (s) spent in social zone}}{\textbf{Total time (s) spent in social and non-social zones}}$$

The number of seconds the focal fish was observed in the 'Neutral' zone was excluded.

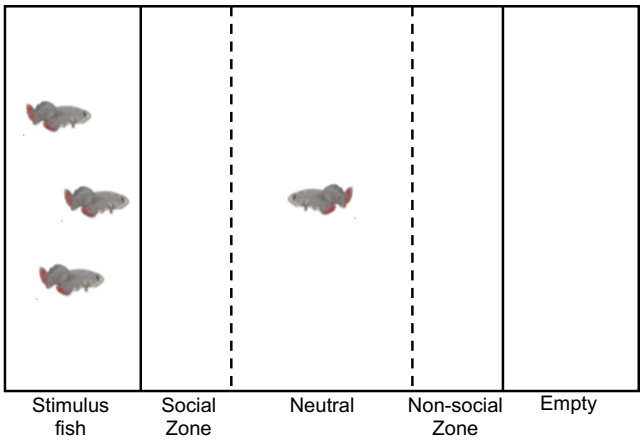


Figure 2: 2D view of the experimental tank (45 x 30 x 27.5 cm). The focal fish was observed in the main compartment (comprising the social (7.5 cm wide), neutral (15 cm) and non-social zones (7.5cm)), whilst three stimulus fish were housed in one of two side compartments (7.5 cm).

2.3.1 Statistical Analyses

Statistical analyses were performed using the R statistical package (R Core Team, 2017), with a significance threshold of α = 0.05. Unless stated otherwise, standard length measurements in this experiment are reported as the mean ± standard deviation (SD) and statistical parameter estimates as the mean ± standard error (SE). Due to the data being proportional (bound between 0 and 1) and zero-inflated, beta regression was used. Prior to analysis, the data transformation $(\boldsymbol{y} \bullet (\boldsymbol{n} - \mathbf{1}) + \mathbf{0.5})/\boldsymbol{n}$ was used as recommended by Smithson and Verkuilen (2006) and Cribari-Neto and Zeileis (2010) to allow analysis of 0 and 1 values, where *y* is preference and *n* is sample size. This removed the extreme values and modified the data accordingly.

Data were analysed using a generalised linear mixed model implemented in the glmmTMB R package (Brooks et al., 2017). The error family was altered to beta and the link function to logit. Under the logit transformation, a proportion of 0.5 corresponds to an intercept of zero, which is therefore the value used to test whether the observed proportion differs significantly from 0.5. Results are reported as; intercept, mean ± SE, Z-value and p-value. The intercept indicates the direction of the preference; a positive intercept indicates a preference for conspecifics, whilst a negative intercept indicates a preference to remain solitary. Preference of the focal fish was tested, using age as a fixed affect, with tank ID

as a random effect to control for non-independence of test fish housed together. Preference for grouping was inferred where the mean preference was significantly greater or less than chance levels (0.5).

2.3.2 Results - Experiment 1- Standard Binary Choice tests

Results from the standard binary choice tests indicate that juveniles do not show a preference to group. Time spent grouping, within the social zone of the main compartment, was not significantly different than expected by chance (GLMM: intercept = 0.061 ± 0.098 [mean ± SE], Z_{117} = 0.625, p=0.532). Similarly, juveniles did not change their social preference with age (0.017 ± 0.05998, Z_{116}=0.028, p=0.782) (Fig 3).

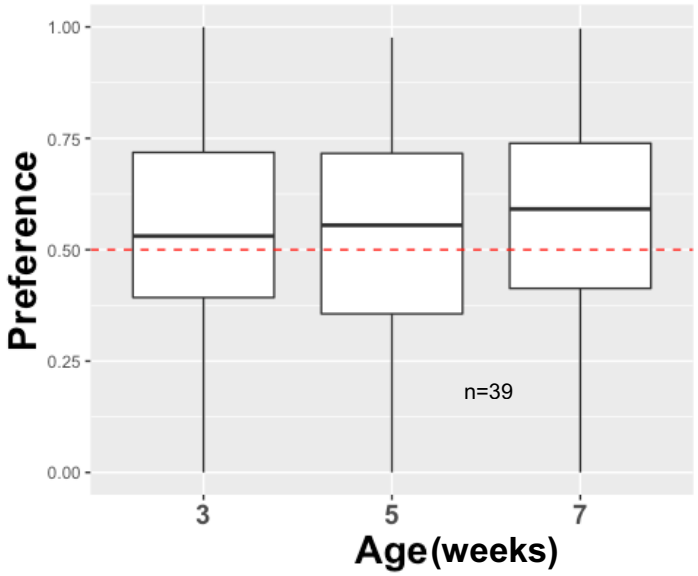


Figure 3: Grouping preference, measured as the proportion of time spent in the social zone of the main compartment, in killifish tested at 3, 5 and 7 weeks of age. Boxplots show the median, interquartile range and maximum and minimum values. The dashed red horizontal line indicates no preference at 0.5.

2.3.3 Discussion – Experiment 1 – Standard Binary Choice tests

The results from the standard binary choice tests suggest at 3, 5 and 7 weeks old, African turquoise killifish are not social. Fish showed no preference for either the stimulus fish in the side compartment of the binary choice tank, nor the side compartment that remained empty. This suggests that they have no preference for either group or solitary living.

Preference to group is complex and depends on both the social and environmental factors influencing it, such as competition for food, increased predation threat and changing habitat conditions (Miller & Gerlai, 2011). Similarly, the age of an individual is likely to influence its propensity to be social. As individuals age, the costs and benefits of grouping change. Young individuals may not experience the same pressures as adults and their propensity to group will reflect this. If group living is only important when killifish are sexually mature, for maximise reproduction in a short timeframe, it is not necessary for juvenile killifish to group.

Grouping is a common anti-predator adaption in several fish species; guppies from populations in which predation risk is high are more likely to form tighter, larger groups as a protection mechanism (Huizinga et al., 2009), red-bellied piranhas (*Pygocentrus nattereri*) form larger groups when at risk from aerial predation (Queiroz & Magurran, 2005), and banded killifish form groups to evade and therefore decrease the chance of individual capture by white perch (*Morone americana*) (Morgan & Godin, 1985).

It is probable that African turquoise killifish are susceptible to predation, yet due to the limited literature on the species the degree of predation pressure they experience is unknown. Tilapiines (*Tilapia*) and catfish (*Siluriformes*) have been observed in pools that contain African turquoise killifish, yet, to date, there has been no literature on either their co-existence with the killifish, or predation events (Polačik & Janáč, 2017). If predation of either terrestrial or aquatic form was low, then the killifish may have no need to form tight, close-proximity groups, which could explain their lack of grouping behaviour. Furthermore, their wild conditions also vary greatly from their laboratory conditions. Under laboratory conditions these fish are given a constant food supply and have no predation risk, therefore, depending on whether anti-predator behaviour is plastic or genetically fixed, their social preference could reflect this.

A possible limitation of the results reported here is that, to date, no-one has determined at what distance African turquoise killifish are considered to be in a group. In this experiment, I used Pitcher's (1983) elective group size (EGS) criterion and measured social interactions as focal fish being within 4 body lengths of the stimulus group. Despite many studies adopting Pitcher's (1983) EGS criterion (see Magurran, 1986; Krause et al., 2000), other authors have – equally subjectively – suggested different EGSs in which fish are considered to be being social, such as 5 body lengths (Viscido et al., 2004), and 7 body lengths (Budaev, 1997; Miller & Gerlai, 2011).

These discrepancies raise the question of whether different species have different EGS criteria for forming groups and whether the standard 4 body lengths, based on Pitcher et al. (1983), can be applied across multiple species as a mechanism to identify grouping tendencies (Miller & Gerlai, 2011). According to Miller and Gerlai (2008) and (2011), Pitcher's criteria has no empirical basis as a limiting distance for communication. This criteria is used under the assumption that to be within a group, fish must be able to communicate with one another (Miller & Gerlai, 2011), and therefore indicates that to communicate, fish must be within 4 body lengths of one another. Different species use different methods and have varying capabilities in communication, their habitat preferences also affecting this. Due to this wide range of both communication methods and habitats it seems rather arbitrary to have a single measure that encompasses all of these grouping tendencies. Therefore, though the standard binary choice test, using Pitcher's (1983) criteria, is most commonly used when observing social preferences in fish where inter-individual grouping distance, or what cues they use to group, is known, if nothing is known about this species' behaviour, this method could be considered unreliable in trying to disentangle what is social and solitary behaviour.

To further this critique, in the more popular model fish species that have been examined for their social tendencies, the availability of visual and olfactory cues (Partridge & Pitcher, 1980), seem to be the essential mechanisms needed to maintain social behaviour. At all time-points during the binary choice tests these cues are readily available; despite being in the 'non-social' zone of the main test compartment, focal fish are in visual contact with the stimulus fish and if they perceive predation risk to be low there may be no need to be in such close

proximity. As there is nothing known about how African turquoise killifish group with conspecifics and the social information they use when making social decisions, creating a test arena in which the use of these cues is more constrained may provide a better method to test their social preference. One possibility is to set up a binary choice test in which the fish must choose which compartment they want to enter, and once they have entered, they lose visual contact with the other compartment.

To implement this possibility, I used the Y-maze as an alternative to the standard binary choice test. The Y-maze experiment was conducted at 7 weeks, which is the same age as the third standard binary choice test, to allow direct comparison with the result of the standard binary choice test. The Y-maze is an established test arena used in many disciplines within fish behaviour and ecology (Engström-Öst et al., 2006; Natt et al., 2017; Ford et al., 2018). Its wide range of usage in the literature suggest it is a reliable model to investigate behavioural choices but, to the best of my knowledge, this is its first use to assess grouping tendencies.

2.4 Experiment 2 - Y-Maze Choice Test

In this experiment, I used 38 individuals that were previously tested in the binary choice experiments. The experimental tank was a white perspex Y-maze filled with 3 cm water at 23.5 °C ± 0.11 (Fig 4). Each arm contained a separate section (7 x 8 x 8 cm) which contained either stimulus fish or was left empty. These were separated from the focal fish by transparent perforated plastic allowing both visual and olfactory contact between the focal and stimulus fish. Each focal fish was contained in the acclimation zone for 2 minutes, and stimulus fish were added at

the same time (see section 2.3 for more details). During the two-minute acclimation time stimulus fish were in both visual and olfactory isolation from focal fish. Once the barrier was removed from the acclimation zone the focal fish was then observed for 10 minutes and the time spent in each arm was recorded. The side of the stimulus groups was alternated between trials to control for a lateral arm bias.

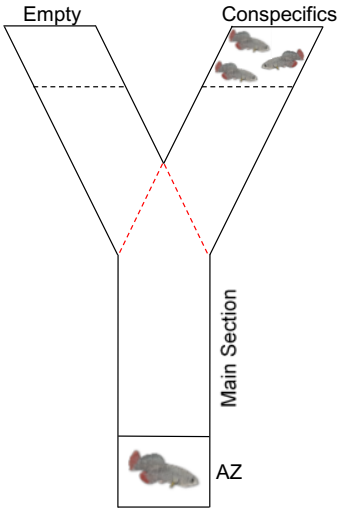


Figure 4: 2D view of the Y-maze experimental design with a main section (27 cm) and two arms (outer length 25 cm, inner length 12 cm, channel width 8 cm). Before the test started, the focal fish was kept in an acclimation zone (AZ) for two minutes. The red dotted lines indicate the point at which a fish was considered to have entered an arm. The black dotted lines indicate the stimulus compartments, one of which contained three conspecifics.

Preference for grouping was measured as the proportion of time spent being social as opposed to being solitary, calculated as:

$$\frac{\textbf{Time (s) spent in arm containing stimulus fish}}{\textbf{Total time (s) spent in either arm}}$$

The number of seconds the focal fish was observed in the main section was excluded. Fish that solely spent time in the main section were similarly excluded from analyses (n=1). All measurements were collected via the same set up as the standard binary choice tank, from a camera positioned above, thus avoiding disruption (see section 2.3 for more details). In this study, killifish were 7 weeks old and on the verge of sexual maturity. Sex could still not be determined unambiguously, therefore the term juvenile is still used. This experiment tested whether, at 7 weeks old, juvenile killifish showed a preference to group.

2.4.1 Statistical Analyses

Results were analysed using the same procedures as in the standard binary choice tests (Section 2.3.1).

2.4.2 Results - Experiment 2 – Y-Maze Choice Test

Results from the Y-maze indicated that killifish showed a preference to be social. Fish spent significantly more time in the arm containing the conspecifics than the empty arm (intercept = 0.550 ± 0.152, Z_{35} = 3.623, p=0.000291) (Fig 5).

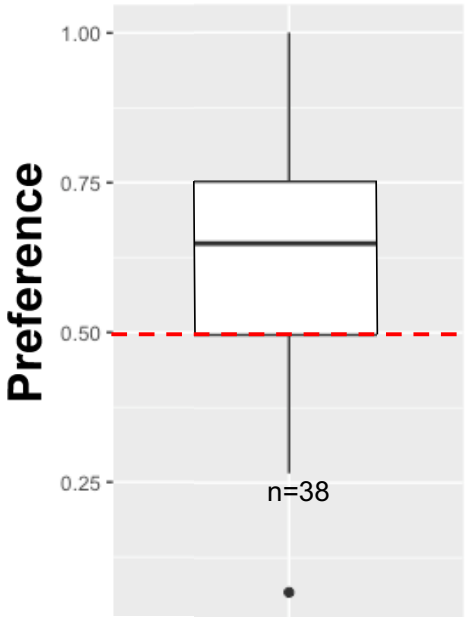


Figure 5: Preference in the Y-maze choice test, measured as the proportion of time spent in the arm containing stimulus fish rather than the empty arm. The boxplot shows the median, interquartile range and maximum and minimum values. The dashed red horizontal line indicates no preference at 0.5. Outliers are any values that are more than 1.5x the interquartile range above the upper quartile and below the lower quartile and are represented by a dot.

2.4.3 Discussion – Experiment 2 – Y-Maze Choice Test

These findings indicate that by 7 weeks of age, African turquoise killifish are social. Whereas in the standard binary choice test, preference to associate was non-significant, in the Y-maze preference for conspecifics was found to be significantly greater than expected by chance. Fish were of the same age and

tested against fish from the same tank, thus the most likely explanation for the difference in results is the loss of visual contact when a focal fish enters an arm in the Y-maze.

The Y-maze's previous usages range from testing the preference/aversion to a particular colour or strobe rate of light in the white sturgeon (*Acipenser transmiontanus)* (Ford et al., 2018), predator-prey interactions in ambion damselfish (*Pomacentrus ambionensis*) and dusky dottybacks (*Pseudochromis fuscus)*, and how these change when background colours vary due to climate change (Natt et al., 2017) and environment choice in three-spined sticklebacks (*Gasterosteus aculeatus*) (Engström-Öst et al., 2006). Using stimulus fish, it has been used to test chemosensory responses of fry (midas cichlids (*Amphilophus citrinellus*)) and their preference for adult males and females (Barnett, 1982). This study used the Y-maze to test the social preference in juvenile African turquoise killifish and highlights the importance of visual contact to killifish when they are grouping. The success of this experiment suggests that the Y-maze could be used in future studies to assess whether a species has a social preference.

2.5 General Discussion

In this chapter, I investigated whether juvenile African turquoise killifish were social. For this purpose, I created two different experimental designs to test grouping preference. The first, the standard binary choice test, is the most commonly used experimental set up when investigating social preference but has limitations due to the current lack of information on the African turquoise killifish (see section 2.3.3 for more details). In this experiment, focal fish were in visual

contact with stimulus fish in all parts of the test arena, despite being in zones considered to be non-social. With no knowledge on the inter-individual distance killifish may consider themselves to be grouping, this raises questions over the suitability of this method. The second experiment, the Y-maze, is an established set-up for other behavioural experiments but this was its first use investigating social preference. This experimental design solves the issue of maintained visual contact, as once fish have entered an arm, they are unable to view the other. Furthermore, the distance between the stimulus groups is much greater. When choosing an arm of the Y-maze, a focal fish is required to swim a further distance than that of the standard binary choice test. These two factors, the loss of visual contact and the length the focal fish must swim to choose a stimulus shoal, suggest that the choice is more definitive and reliable in the Y-maze set-up. In the Y-maze choice test, but not the standard binary choice test, juvenile killifish preferred to group rather than remain solitary.

2.5.1 Grouping Preference

Grouping, in fish, confers many benefits, such as anti-predator defences (Krause & Ruxton, 2002), increased mating opportunities and greater foraging efficiency and activity (Pulliam, 1973; Wolf, 1987). The killifish in this experiment were not yet sexually mature and therefore it is unlikely that their groups were related to mating opportunities. Similarly, as discussed in section 2.3.3, the degree of predation threats African turquoise killifish face, is unknown. Finally, there is not yet any information on African turquoise killifish foraging behaviour. In the laboratory, they are fed brine shrimp and bloodworm, yet their diet in the wild is unknown. Social groups could potentially improve juvenile foraging success, through increased vigilance if predators are apparent (Pulliam, 1973), greater

information transfer on the location of food (Pitcher et al., 1983) and quicker time to find food (Pitcher et al., 1982). This might explain why, as well as potential protection from predators, they are forming groups.

2.5.2 Future Research

There is a large ontogenetic variation in the timing and tendency of when fish species choose to group and who they group with. Though grouping in juveniles has now been observed, the preference of adult African turquoise killifish is still unknown. Adult preference is further modulated by preference for potential partners, size and colouration of the group and possible predation risk due to increased size and visibility. This therefore leads to more questions about how African turquoise killifish are socially composed within their pools and whether this changes with age and selection pressures.

There are no previous experiments investigating the grouping preferences of the African turquoise killifish. This is due to the literature being severely lacking on both the behaviour and ecology of this species. Unfortunately, I have nothing to compare the sociality of this species to, it could be a species-wide behaviour or one that is limited to certain strains. This study is, to my knowledge, the first to investigate African turquoise killifish grouping tendency, so my main aim was simply to establish whether these fish were social. Kinship and familiarity were not accounted for, nor was the strain of the fish. Sociability could, in principle, vary between strains and this is something future researchers should address.

More is known about the behaviour of other killifish species. Mangrove killifish (*Kryptolebias marmoratus*) behaviour has been studied extensively by Edenbrow *et al.* who showed that mangrove killifish are more exploratory and bold when juvenile and once adults they reduce these traits (Edenbrow & Croft, 2011). Their social experience when juvenile influences behavioural expression when older and also influences their preference for familiar and related individuals (Edenbrow & Croft, 2012b; Edenbrow & Croft, 2013). Replicating these studies on the African turquoise killifish would provide further information about their grouping tendencies. In the present study, I have shown that juveniles show a preference for other individuals, therefore a preference to group. However, the composition of these groups is as yet unknown. There are associated benefits to socialising with kin and familiar conspecifics (see Ward & Hart, 2003 for review), and due to the fact that African turquoise killifish live in ephemeral pools that do not overlap, thus limiting dispersion, it is likely that they will be familiar and related to a large proportion of the conspecifics they encounter.

In the next chapter (Chapter 3) I further examine the social behaviour of the African turquoise killifish by exploring both juvenile and adult social preferences. Using the Y-maze, I perform the same choice test from this chapter on juveniles and adults from the F3 generation. This enables me to see whether adult killifish have a social preference and whether this differs from juveniles. I also investigate whether juvenile and adult African turquoise killifish are capable of recognising kin and familiar individuals. This study then provides us with an opportunity to see whether these behaviours change as an individual ages, thus, observing the shifts in behaviour over a lifespan.

2.6 Conclusion

This study has addressed for the first time whether juvenile African turquoise killifish have a preference to group with conspecifics. Using two test arenas, I investigated which better suited this study system and determined their grouping tendencies using the most appropriate set-up. Using the Y-maze, I established that juvenile African turquoise killifish are social by 7 weeks of age. Further research is needed to understand what mechanisms underlie this sociability and whether preference is biased towards related or familiar individuals.

Chapter 3: The effect of age, sex, familiarity and kinship on social preferences in the African turquoise killifish

3.1. Introduction

Group living is one of the most prominent and widespread aspects of animal behaviour (Hoare et al., 2000; Krause & Ruxton, 2002), the benefits of which are well-documented across a range of taxa and include increased access to mating opportunities (Monaghan & Metcalfe, 1985), foraging activities (Baker et al., 2004), and greater anti-predator defence (Hass & Valenzuela, 2002). In fish, this behaviour is extremely common, with approximately 25,000 species forming groups at some point in their lives (Shaw, 1978; Wright & Krause, 2006).

It is now widely accepted that fish groups are not randomly composed and differ depending on the species of fish and life-history adaptations, as well as external stimuli such as predator threats and habitat type (Griffiths & Magurran, 1999; Pavlov & Kasumyan, 2000). Grouping requires recognition, at the most basic level, of species, body size and other phenotypic traits (see Ward & Webster, 2016 for a review on recognition). Recognition provides animals with information about their group members and individuals capable of recognition have access to more specific benefits. In particular, recognition of familiar individuals is beneficial and known to reduce aggression (Höjesjö et al., 1998; Utne-Palm & Hart 2003), improve information transfer (Swaney et al., 2001), and mediate mate choice (Ward et al., 2002; Ward & Hart, 2003) and has been demonstrated in guppies (*Poecilia reticulata*) (Griffiths & Magurran, 1999), fathead minnows (*Pimephales promelas*) *(Chivers et al., 1995)*, European minnows (*Phoxinus*

phoxinus) (Barber & Wright, 2001), and angelfish (*Pterophyllum scalare*) (Gómez-Laplaza, 2006), amongst others.

Similarly, individuals that are capable of kin recognition can bias co-operative behaviours and direct them at individuals dependent on their genetic relatedness. By biasing their behaviours in this way, individuals increase their inclusive fitness through increasing the survival and reproduction of genetic relatives. Additionally, kin recognition decreases the chance of cannibalism (Fitzgerald & Morrissette, 1992) and increases inbreeding avoidance (Frommen & Bakker, 2006).

Kin recognition has been demonstrated in numerous fish species including Arctic charr (*Salvelinus alpinus*) (Olsen et al., 1998), zebrafish (*Danio rerio*) (Gerlach & Lysiak, 2006), coho salmon (*Oncorhynchus kisutch*) (Quinn & Busack, 1985), three-spined sticklebacks (*Gasterosteus aculeatus*) (Fitzgerald & Morrissette, 1992), and mouth-brooding tilapia (*Sarotherodon melanotheron*) (Pouyaud et al., 2002; Ward & Hart, 2003). Its benefits include increased growth in cichlid fish (*Pelvicachromis taeniatus*) (Thünken et al., 2016), increased foraging benefits in Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) (Brown & Brown, 1996) and are further discussed in Ward and Hart (2003).

Interestingly, the mechanisms behind kin recognition and familiarity vary depending on the species and reflect the social environment in which they live. Familiarity can be learned via prior association, through inhabiting the same environment or natal area. After spending a large amount of time with other group

members, the individual gains the ability to discriminate them from others they have not associated with (Ward & Hart, 2003 for review). In contrast, kin recognition is possible without any prior association. Visual, olfactory, acoustic and genetic cues are used by individuals to phenotypically match themselves to others (Gerlach & Lysiak, 2006), enabling them to identify others without any previous encounters or knowledge about the individual.

Though both kin recognition and familiarity have been well-researched in fish biology, there is a large gap in our knowledge on the critical components that influence the onset and modification of recognition as individuals age. It is likely that ontogenetic changes in life-history strategies influence recognition and the reasons for recognising others. Juveniles are often subject to different social and ecological conditions than adults and their need and ability to recognise other individuals might therefore be different.

Previous work on grouping, kin recognition and recognition of familiar individuals have used fish at specific points during their lifespan, at either a juvenile or adult stage (see Frommen & Bakker, 2004; Behrmann-Godel et al., 2006; Gerlach et al., 2008 for examples). Due to their relatively long lifespan, many traditional laboratory fish are not suitable for ontogenetic studies. The short lifespan of African turquoise killifish could provide an insight into the behavioural changes that occur as an individual ages.

This chapter aims to investigate whether both juvenile and adult African turquoise killifish are capable of grouping and if so, whether this preference is biased

towards related or familiar individuals. In chapter 2, I looked at grouping in juveniles, changing the experimental design to suit the information known about African turquoise killifish. In this chapter, my aim is to investigate how juveniles and adults differ in their preference for conspecifics, which would indicate that preference changes with age.

I predicted that juvenile and adult African turquoise killifish would group, yet would differ in their preference to group, due to their contrasting life history adaptations. It is possible that smaller fish such as juveniles are more vulnerable to predation, therefore their propensity to group may be greater than adults. Contrastingly, adults may group for reproductive reasons therefore they may be more likely to show a preference for grouping than juveniles. Due to their sexual dimorphism and dichromatism, males may be more susceptible than females to predation and grouping may provide them with more benefits than solitary living.

I also wanted to investigate whether adult males and females differ in their preference for grouping with kin and familiar individuals. It is possible that due to their habitat preferences, which cause limited dispersal, wild African turquoise killifish could be in close contact with familiar and related individuals. It is likely that there will be benefits from these interactions, such as avoiding inbreeding and also reducing aggression. Therefore, in this experiment I predicted that I would see a preference for this.

3.2. Methods

3.2.1 Breeding individuals

Fish were purchased from Aquasense LTD (Bucks, UK) and despite being commercially available, their population origin is unknown. A breeding population of fish was established and individuals from the F2 generation were split into breeding pairs, creating 7 tanks (27 (length (L)) x 18 (width (W)) x 18 (depth (D)) cm), each with a male and female housed inside. Tanks were labelled with the ID of both the male and female within and were supplemented with a refuge and small spawning tanks (8L x 8W x 8D cm) filled with peat (Verve multi-purpose compost). Pairs were left for a week to settle and begin spawning and once this had begun, eggs were collected at 2-4 day intervals until there was a sufficient number for hatching. As adults, fish were fed a mixed diet of brine shrimp nauplii (*Artemia salina)* and bloodworm (*Glycera dibranchiata*) daily. Eggs were labelled according to the ID of both parents with only eggs from the same pairing incubated together. Once mature, eggs were hatched in separate containers according to parent ID and then transferred to corresponding tanks.

3.2.2 Experimental subjects

From hatching, juvenile fish were kept in groups of 7 individuals in transparent aquaria (22L x 13W x 13D cm), which were visually isolated from other group tanks. The individuals within a tank were full siblings but the sex of each fish could not be determined, so the sex ratios at this stage were unknown. Temperature was maintained at 24.31°C ± 0.12 (mean ± standard deviation (SD)) and illumination was set on a 12:12 light:dark cycle. Fish were fed uniform pipettes of brine shrimp nauplii in a randomised order to avoid a feeding bias and water changes were carried out at 2-week intervals.

At 6 weeks old, after the juvenile experiments (see below) and prior to sexual maturation, fish were transferred into larger home tanks (27L x 18W x 18D cm) due to their size. Refuge and small spawning tanks (8L x 8W x 8D cm) were added once fish became sexually mature. Aeration via sponge filters was introduced to remove debris and food remains and maintain tank cleanliness. When the fish were adults, water changes were carried out every 7 days.

This experiment used 3 focal individuals from each tank of 7, each tested once per experiment. All testing took place within a 1 week period. Focal fish were kept overnight in separate tanks containing a 50:50 mix of water from their home tanks and new water. Focal fish tanks were placed adjacent to their home tanks containing the stimulus fish, allowing for both visual and olfactory contact (via the addition of their home water) to be maintained. After the juvenile experiments focal fish were returned to their home tanks, without being tagged (due to their age and size); therefore, when choosing focal fish for the adult experiments, it was unknown whether these fish had been focal fish as juveniles. Between experiments there was a 3-month break, so any effects of being a focal fish or stimulus fish should not have made a difference in the adult experiment, due to the large amount of time that had passed. Any fish that died were removed from the tank and the remaining fish were left in their group with no other fish added. Fish were originally placed in groups of 7 within a tank, yet due to the mass mortality, in the adult experiments, group sizes ranged from 4 – 7 individuals. In both the juvenile and adult experiments, focal fish were not used as stimulus fish and vice versa. Experiments were conducted when juveniles were 5 weeks old (1.43cm ± 0.29) and when adults were 17 weeks old (2.94cm ± 0.48).

Upon hatching and at 3-week intervals, groups were transferred to a holding tank (22L x 13W x 13D cm) and photographed from above using a Panasonic HC-V750 camcorder. Measurements were taken to assess growth and health and size was measured from jaw to caudal peduncle using Image J photo analysis software (Rasband, 2017). This data was inputted into the model and used as a predictor to determine whether size affected preferences. In the adult experiments, size and sex of the focal fish was measured and could be matched to the video analysis. This was not possible in the juvenile experiments as sex of the fish was unknown and could not be determined unambiguously.

3.2.3 Experimental set-up

In the previous chapter I discussed the merits of using a Y-maze instead of a standard binary choice test. In African turquoise killifish the distance between two fish considered to be grouping is unknown, therefore a standard binary choice test is unsuitable. Using a Y-maze design solved the issues presented by the standard binary choice test and allowed me to investigate grouping preferences reliably. For this reason, its usage has been continued in the forthcoming experiments. Y-mazes were made using white Perspex and were size-matched to both juveniles and adults (Fig 1). Each arm was separated by transparent perforated plastic (represented by the dotted lines at the ends of both arms in fig 1) and contained either familiar kin, unfamiliar kin, unfamiliar non-kin or remained empty. This allowed both visual and olfactory contact between the focal fish and the stimulus fish.

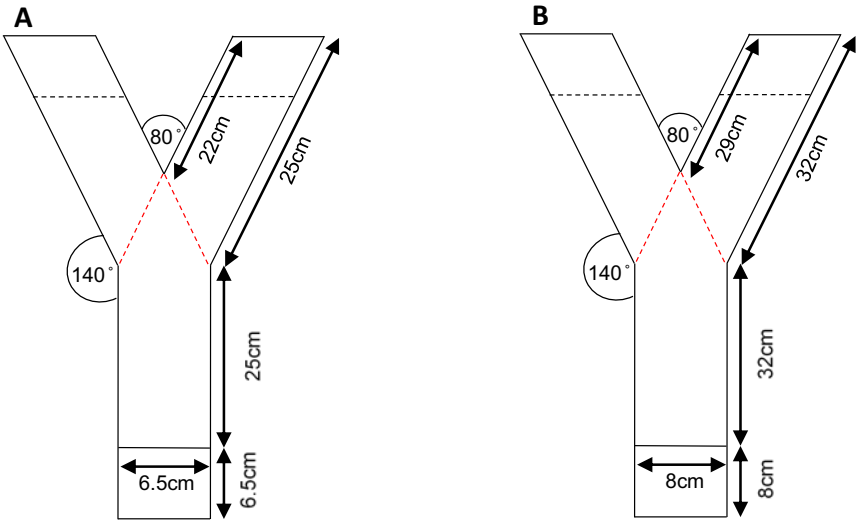


Figure 1: A) Y-maze used for juvenile testing. Width and length correspond to body length measurements taken from the F2 population: width was approximately 4 x the body length of the focal fish, whilst length was approximately 4 x the maze width. B) Y- maze used for adult testing; measurements were taken in the same way. The depth of each Y-maze was 8cm.

To investigate preference for conspecifics, kin and familiars, four main tests were used. Each focal fish was allowed to acclimatise in a compartment in the main section for two minutes (indicated in fig 1 as the box on the left-hand side at the base of the Y-maze). This compartment was not perforated and was visually isolated from the stimulus compartment, thereby precluding olfactory and visual contact with stimulus fish that were added at the same time. The focal fish was then released and the time it spent in each arm was recorded. Experiments were 10 minutes long and after each experiment the water was changed. To avoid a lateral arm bias, the side of the stimulus fish groups was alternated. For grouping preference, one arm of the Y-maze contained conspecifics that were both familiar and related to the stimulus fish, whilst the other remained empty (Juveniles (J): n

= 51, Adults (A): n = 35). Grouping preference was measured as the number of seconds the fish spent in the arm containing the conspecifics, as a proportion of the number of seconds the fish spent in either arm of the Y-maze.

To examine whether focal fish have a preference for kin and familiars, three tests were completed. In the first experiment, one arm contained familiar kin, whilst the other contained unfamiliar non-kin (J**:** n = 50, A**:** n = 34). This experiment tested a preference for familiarity and relatedness over unfamiliar, non-related fish. In the second experiment, one arm contained familiar kin, whilst the other contained unfamiliar kin (J**:** n = 48, A**:** n = 24). This experiment tested the stimulus fish preference for familiarity. In the third experiment, one arm contained unfamiliar kin, whilst the other contained unfamiliar non-kin (J**:** n = 48, A**:** n = 22). This experiment tested preference for related individuals.

To statistically analyse preference for kin and familiarity some tests were combined. Preference for familiarity was measured as the number of seconds the fish spent in the arm containing the familiar individuals, as a proportion of the number of seconds the fish spent in either arm of the Y-maze. Preference for kin was measured as the number of seconds the fish spent in the arm containing the related individuals, as a proportion of the number of seconds the fish spent in either arm of the Y-maze.

3.2.4 Statistical analysis

The statistical package R (R Core Team, 2017) was used to perform statistical analyses, with a significance threshold of α = 0.05. Unless stated otherwise, standard length measurements in this experiment are reported as the mean ±

standard deviation (SD) and statistical parameter estimates as the mean ± standard error (SE). Due to the preference data being bounded by 0 and 1, the following data transformation was implemented prior to analysis: $(\mathbf{y} \bullet (\mathbf{n} - \mathbf{1}) + \mathbf{0.5})/\mathbf{n}$, where *y* are the original proportional values and *n* is the sample size. The transformed data were analysed using a generalised linear mixed model in the glmmTMB R package (Brooks et al., 2017), with a beta error distribution and a logit link function. The logit function transforms a proportion of 0.5 to a value of 0, thus testing whether the intercept differs significantly from 0 is equivalent to testing whether the mean preference differs significantly from 0.5. A negative intercept indicates a proportion of <0.5 whereas a positive intercept indicates a proportion of >0.5.

Preference of the focal fish was tested against a range of main predictors (Table 1), with tank ID (of the tank in which the focal fish was housed when not being tested) always added as a random effect. Preference for any of the predictors was inferred where the mean preference was significantly different from 0.5. Continuous variables were standardised so that their mean = 0 and the standard deviation was 0.5, making them directly comparable despite being measured on different scales (Schielzeth, 2010). The binary variables ‘kin’ and ‘familiarity’ were left unstandardized following (Gelman, 2008). Global models were created with all of the predictors of interest and interaction terms present. Only one interaction term was used: to examine whether preference for kin or familiars was modulated by female density (number of females in the stimulus group). Home group size (of the group in which the focal fish was housed) was added as a predictor in the adult experiments due to mass mortality in multiple tanks. Despite all tanks starting with 7 individuals inhabiting them, disease reduced many of these

numbers, hence ratios of males and females in the stimulus shoals differed between groups; ratios were not systematically varied. Disease occurred in early life between juvenile and adult experiments. Disease caused rapid deterioration of health and ultimately death of the affected fish within 2-3 days. Two months after the outbreak there were no further deaths and fish were cleared of disease. Fish were deemed healthy and were permitted to be used in these experiments. Fish used in adult experiments came from tanks ranging from 4 to 7 individuals. This predictor was therefore used to account for this and to determine whether group size affected preference to be social and influence kin or familiar choices.

Adult preferences were analysed using a model inference and averaging approach. This was not possible for juvenile analysis as certain predictors such as the sex and the size of the focal and stimulus fish could not be measured; sexual dimorphism and dichromatism does not develop until later in life and the identity of the individuals between experiments could not be determined accurately, thus making size measurements irrelevant. In the adult preference experiments females and males were analysed separately due to the dimorphism and dichromatism between the sexes. Using the dredge function in the MuMIn R package (Barton, 2018), every possible model was compared and a set of best-supported models were chosen based on AICc (Akaike information criterion (Akaike, 1973; Burnham & Anderson, 2003)) values, the most widely used criterion in the model inference and averaging literature (Grueber et al., 2011). Models within <2 delta AIC (ΔAIC_c) were considered statistically indistinguishable and are shown in table format in the results section. All model weights were <0.9, so model averaging was recommended (Grueber et al., 2011). The model averaging function in the MuMIn package was used to obtain parameter values

that were averaged across the best models identified in the dredge package. The summary of values presented in tables in the results section are from the 'full average' output, which includes parameter estimates of zero (following Nakagawa and Freckleton (2011). The only result not to have undergone the model averaging approach was male preference for grouping, where the null model was the best model based on ΔAIC_c and no other model values came within 2 ΔAIC_c. For grouping preferences in the adult fish, heritability was estimated using ASReml-R (Butler, 2009). To investigate whether grouping preference is a heritable trait, I estimated the narrow-sense heritability (h^2) of grouping preference across phenotypes with the size of the focal fish and its sex as fixed factors. The heritability of related and familiar preferences could not be performed due to the lower sample sizes.

Table 1: Description of the predictor variables used to assess killifish social preferences

Predictor variable	**Description**
Kin	Choice between kin and non-kin (experiments testing *familiar kin vs non-kin* and *non-familiar kin vs non-kin*)
Familiarity	Choice between familiar and unfamiliar individuals (experiments testing *familiar kin vs unfamiliar non-kin* and *familiar kin vs unfamiliar kin*)
Home group size	The number of fish present in the focal fish's home tank, varying from 4-7 individuals
Female density	Difference in the number of females between the two stimulus groups, calculated as: number of females in the familiar/kin group minus number of females in the

	unfamiliar/non-kin group. A negative value indicates that there are more females in the unfamiliar/non-kin group, whereas a positive value indicates that there are more females in the familiar/kin group.
Length	Body length of the focal fish being tested
Tank ID (random effect)	Information about which tank the focal fish came from, which was categorised by their parents' IDs

3.2.5 Ethical note

This research was approved by the Psychology ethics committee at the University of Exeter (ref. 2017/1658). Fish used were bred from the F2 generation of laboratory fish, meaning no transport of experimental fish was necessary. Between juvenile and adult experiments the killifish experienced mass mortality. Fish were sent for a health screening and results found velvet disease and fish tuberculosis. Appropriate measures were taken to minimise the spread of diseases and fish were checked daily for signs of deterioration. Two months after diagnosis fish were healthy and permitted to be tested on. There was no physical or invasive manipulation as these were behavioural assays.

3.3. Results

3.3.1 Juveniles

3.3.1.1 Grouping

The mean preference for grouping (preference for the arm containing the stimulus fish) was 0.603, which was not found to be significantly different from 0.5, indicating no preference for either grouping or being solitary (GLMM: intercept = 0.283 ± 0.203 [mean ± SE], Z_{50} = 1.39, p = 0.165). This indicates that at 5 weeks old, juvenile killifish are not yet social.

3.3.1.2 Kin recognition and familiarity

Similarly, when investigating kin recognition and familiarity, juvenile killifish had no preference for either individuals that were related, or individuals that were familiar to them (Table 3).

Table 3: Model estimates and SE for fixed factors when analysing juvenile killifish preference for kin and familiars. Tank ID was included as a random factor

Parameter	***Estimate ± SE***	***z-value***	***p-value***
Intercept	0.412 ± 0.327	1.259	0.208
Kin	-0.309 ± 0.258	-1.196	0.232
Familiarity	-0.204 ± 0.259	-0.790	0.430
Random effect			
tank estimated variance = 0.04138			

3.3.2 Adults

3.3.2.1 Grouping

For grouping (the choice between an arm containing stimulus fish and an empty arm), a global model was made containing all of the predictors within this experiment (home group size, female density and length). When investigating female preference for grouping the best model contained female density as a predictor variable. However, this model was not distinguishable from the null model (Table 4), so a model averaging method was needed. Though home group size varied during both the grouping and kin and familiarity experiments, it did not affect the focal fish's preference to be social and was not a predictor in any of the best models determined by dredging.

Table 4: Candidate models used when testing female and male adult preference for grouping. All models included tank ID as a random factor (not shown). Candidate models are ranked, with the highest indicating a better model. k represents the number of fitted parameters. Models were chosen based on lowest Akaike's information criterion adjusted for sample size (AIC_c), and largest Akaike weights (ω). All models in this table had a ΔAIC_c of <2

Test	*Rank*	*Fixed effects in candidate model*	AIC_c	ΔAIC_c	*k*	*ω*
Female	**1**	**~ female density**	-6.06	0	4	0.7
	2	**intercept only**	-4.31	1.74	3	0.3
Male	**-**	**intercept only**	1.3	-	3	-

Model averaging indicated that females show a significant preference to group with conspecifics (Fig. 2). Despite female density being included in the best

model, it did not significantly affect a female adult killifish's preference to group (Table 5). Male preference for grouping was best represented (with the lowest AICc by >2) in the null model that contained no predictors (Table 4). Males spent significantly more time in the compartment containing conspecifics than expected by chance, indicating a preference for grouping (Table 5). To investigate whether this grouping preference was heritable, I used narrow sense heritability (h^2) and accounted for size and sex of the focal fish. Heritability was found to be low and non-significant ($h^2 = 0.173 \pm 0.312$).

Table 5: Model estimates ± SE (adjusted SE for standardised predictors if the model was averaged), z-value and p-values for both female and male adult killifish preference for grouping with conspecifics. Tank ID was included as a random factor. P-values that are significant are highlighted in bold

Sex	***Parameter***	***Estimate ± SE***	***Adj. SE***	***z-value***	***p-value***
Female	**Intercept**	1.119 ± 0.282	0.304	3.678	**<0.001**
	Female density	-0.854 ± 0.696	0.721	1.184	0.236
Male	**Intercept**	0.587 ± 0.274	-	2.144	**0.032**
	Random effect				
	tank estimated variance	< 0.001			

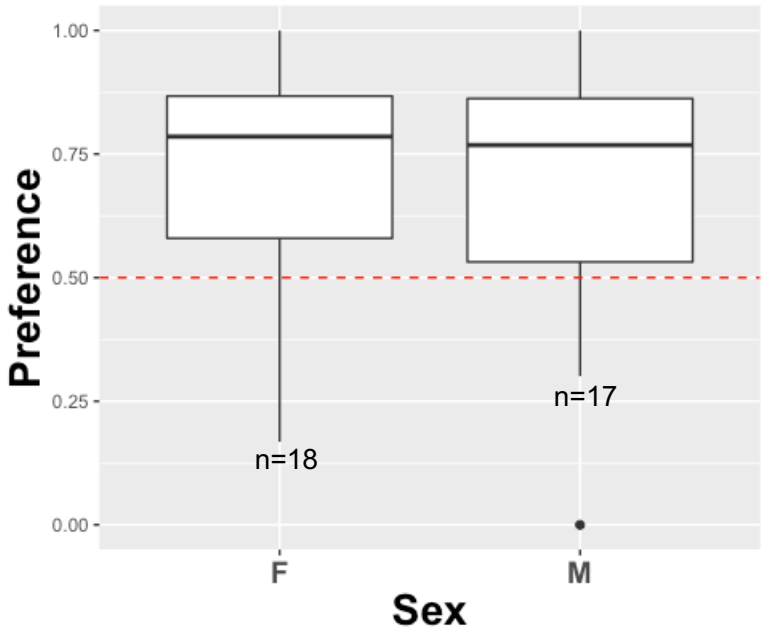


Figure 2: Preference for grouping in both males (M) and females (F), measured as the proportion of time spent in the arm of the Y-maze containing the conspecifics. Both female and male preference for conspecifics was significantly greater than expected by chance. Boxplots show the median, interquartile range and maximum and minimum values. Outliers are represented as dots and are any values that are more than 1.5x the interquartile range above the upper quartile and below the lower quartile.

3.3.2.2 Kin recognition and familiarity

When investigating female and male preference for kin and familiar conspecifics, global models were created using the following predictors: kin, familiarity, female density, and focal fish body length. Interaction terms were added between female density and kin and between female density and familiarity to investigate whether female density modulated the focal fish's preference for kin or familiar individuals.

Table 6: Candidate models used when testing female and male adult preference for kin and familiars. All models included tank ID as a random factor (not shown). Candidate models are ranked, with the highest indicating a better model. k represents the number of fitted parameters. Models were chosen based on lowest Akaike's information criterion adjusted for sample size (AIC_c), and largest Akaike weights (ω). All models in this table had a ΔAIC_c of <2

Sex	***Rank***	***Fixed effects in candidate model***	***AIC_c***	***ΔAIC_c***	***k***	***ω***
Female	**1**	**~ kin**	4.72	0.00	4	0.25
	2	**intercept only**	4.98	0.26	3	0.22
	3	**~ length**	5.21	0.49	4	0.20
	4	**~ kin + length**	5.24	0.52	5	0.20
	5	**~ kin + familiarity**	6.13	1.40	5	0.13
Male	**1**	**~ familiarity + female density + familiarity*female density**	-0.52	0.00	6	0.51
	2	**~ familiarity + female density + familiarity*female density + length**	-0.47	0.05	7	0.49

For females, model averaging indicated that the best model contained kin, but was not distinguishable from the null model, or models containing the predictors length and familiarity (Table 6). Both kin and familiarity predictors were non-significant (Table 7), suggesting that females show no significant preference for either related or familiar individuals. Length of the focal fish was also non-significant, indicating that this did not affect their preference for kin or familiars.

Table 7: Model estimates ± SE (adjusted SE for standardised predictors if the model was averaged), z-value and p-values for both female and male adult killifish preference for kin and familiars. Tank ID was included as a random factor. P-values that are significant are highlighted in bold

Test	***Parameter***	***Estimate ± SE***	***Adj. SE***	***z-value***	***p-value***
Female	**Intercept**	-0.406 ± 0.484	0.494	0.823	0.411
	Kin	0.401 ± 0.465	0.471	0.851	0.395
	Length	-0.423 ± 0.693	0.704	0.600	0.548
	Familiarity	0.061 ± 0.222	0.226	0.268	0.789
Male	**Intercept**	-0.729 ± 0.381	0.393	1.856	0.063
	Familiarity	0.596 ± 0.350	0.363	1.639	0.101
	Female density	0.516 ± 0.522	0.566	0.770	0.341
	Familiarity *Female density	-2.142 ± 0.720	0.747	2.870	**0.004**
	Length	0.436 ± 0.558	0.566	0.770	0.441

Contrastingly, for male preference, investigated through the model averaging approach, two models were distinguishable from the null model, yet not distinguishable from each other (Table 6). Both models contained the predictors familiarity, female density and an interaction between female density and familiarity, while body length was included in one model but not the other. The only significant predictor was the familiarity × female density interaction term (Table 7). As the number of familiar females increases, male preference for familiarity decreases. This suggests a male preference for familiars when they are predominantly male (Fig 3).

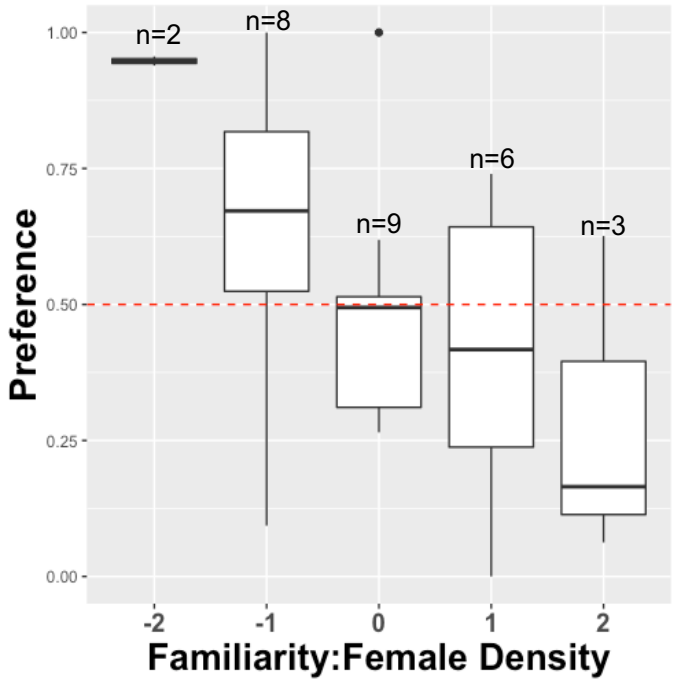


Figure 3: Male preference for familiarity modulated by female density. Female density is defined in Table 1. A negative value here indicates there are more females in the unfamiliar group. A positive value indicates there are more females in the familiar group. e.g. a value of -2 indicates that there are 2 less females in the familiar group than in the stimulus group, which means in the familiar group there is 1 female present and in the stimulus group there are 3 females present. Male preference for familiarity modulated by female density is measured as the proportion of time they spend in the arm of the Y-maze containing familiar individuals and whether this is affected by the number of females present. A significant downward trend was present. Boxplots show the median, interquartile range and maximum and minimum values. Outliers are any values that are more than 1.5x the interquartile range above the upper quartile and below the lower quartile and are denoted as dots. A value of 0.5 indicates no preference.

3.4. Discussion

African turquoise killifish exhibit social preferences that differ with their age and sex. 5 week old juveniles do not preferentially associate with conspecifics and therefore do not show a bias towards kin or familiar individuals. Whereas adults do show a preference for grouping, with both males and females preferring to associate with conspecifics than remain solitary. Grouping preference was not found to be highly heritable, but this may be due to the small sample size, hence the large variance. The preference for kin and familiars differs between the sexes. Females show no preference for either related or familiar individuals. Males, however, despite showing no preference for related individuals, show a preference for other males when familiarity is a factor.

The onset of grouping behaviour and preference for kin and familiarity was previously unknown in African turquoise killifish. Though juveniles showed no signs of grouping tendencies, adults preferred to associate with conspecifics. The ontogenetic shift in grouping behaviour in African turquoise killifish, from juveniles to adults, must confer an environmental relevance. Selection pressures such as mating, foraging and anti-predator defences are known to be amongst the reasons animals form groups and it is possible that as an individual ages these pressures change (see Stratmann & Taborsky, 2013 for an example).

3.4.1 Sociality across a lifespan

Juvenile killifish showed no preference for grouping with conspecifics, related or familiar individuals, which though not common in the literature, is not unheard of. Examples of a lack of grouping preference in other juvenile fish species include

solitary migration which has been previously observed in Atlantic salmon smolts that group later downstream (Riley, 2007), juvenile bridled monocle bream (*Scolopsis affinis*) which are solitary and inhabit sheltered lagoons (Radford et al., 2011), and bluehead wrasse (*Thalassoma bifaciatum*) which are initially found as solitary individuals and group later in their development (Searcy & Sponaugle, 2001).

Numerous species grouping tendencies change as they mature and develop recognition capabilities. Spanish mackerel (*Scomberomorus niphonius*) do not begin grouping until their prey capture capabilities are established (Masuda et al., 2003). Group cohesion changes as zebrafish age, with newly hatched zebrafish forming loose groups, whilst adults form tight groups (Buske & Gerlai, 2011). In another study, juvenile zebrafish showed no preference for groups until an age when they were able to recognise differences in pigment patterns (Engeszer et al., 2007). This preference co-occurs with the sophistication of their visual system and the ability to visually distinguish between conspecifics and heterospecifics.

Unlike juveniles, adult killifish are sexually dimorphic and male colouration is heavily pigmented. If grouping preference is linked to colour, visually mediated associations would not occur until fish were adults and on the basis of sex discrimination, which is important for both mating and reproductive success. Thus, it is not unusual that juvenile killifish do not form groups as being close to potential mates is only a fitness advantage, and therefore only necessary, when individuals are reproductively mature (Pitcher, 1986).

I intended to measure the difference between juvenile and adult killifish in their preference for associating, but this was not possible due to the mass mortality that occurred between experiments. Though the results cannot be statistically compared to establish whether the preference was significantly stronger in adults, this study still shows that juvenile killifish do not preferentially associate with kin or familiar individuals and do not group, whilst adult killifish do show a preference for grouping, with males also showing a preference for other familiar males.

My aim was to investigate the effects of grouping, kin recognition and familiarity on both juvenile (5 week old) and adult killifish. To do this it was necessary to simulate as natural conditions as possible. Due to their habitat it is extremely likely that killifish remain in their natal area for the duration of their lifespan and that the fish surrounding them will be kin. In my experiment, fish were reared in multiple kin groups. Within each kin group, fish were separated into two tanks to create a familiar kin and unfamiliar kin group for the focal fish tested. Unfortunately, this means that when testing familiarity, both stimulus groups were also kin, meaning I am unable to disentangle the two phenomena as they are strongly intertwined. To fully understand what type of recognition is having the largest effect, it would be necessary to include a group of familiar unrelated individuals. Though this is not representative of their environment, it would discriminate between kin and familiar recognition. In addition, this study has focused on a small group of individuals. It is possible that in wild conditions, as group size increases, the ability to recognise familiars decreases. Griffiths and Magurran (1997) have demonstrated this in Trinidadian guppies, whose preference was constrained by group size. It is also possible that as group size

increases, the number of related individuals decreases, therefore the number of other fish an individual is able to recognise also decreases.

3.4.3 The influence of familiarity on grouping preference

These data confirm that males are capable of recognising other familiar related males and preferentially choosing to group with them. As the number of familiar males increases in the stimulus group, focal males are more likely to spend a greater amount of time in this compartment. A large amount of evidence is now present in the literature, demonstrating the benefits of recognising familiars. Fish have been found to be less aggressive towards their familiar counterparts (Utne-Palm & Hart 2003; Olsén & JäUrvi, 2005), form more cohesive groups with greater vigilance (Chivers et al., 1995; Griffiths et al., 2004), and are more exploratory in the presence of a familiar individual (Bhat & Magurran, 2006).

Interestingly, wild populations of adult killifish are significantly female-biased (Reichard et al., 2014), despite their sex being genetically determined (Ewulonu et al., 1985; Reichard et al., 2009). This indicates a significant decline in the proportion of males as killifish age. In my captive population the ratio of females to males was equal, suggesting a strong environmental component of selection. Reichard et al. (2014) found that vegetated sites had more males than sites in which there was turbid water and vegetation was lacking, indicating vegetation cover may have decreased their visibility to predators. Their bright colouration is sexually selected and present in all *Nothobranchius* species (Haas, 1976) and displays to attract females may increase mortality through predator detection. If predation threats are apparent, it is likely that males group for anti-predator

reasons; decreasing the chance of being predated on due to the dilution effect (Foster & Treherne, 1981). Furthermore grouping with familiar individuals has been found to increase cohesiveness of groups (Chivers et al., 1995), which for male killifish, could mean that associating with other familiar males decreases chances of predation further.

Another plausible explanation for males preferring familiar males is aggression. Male-male aggression stems from competition for females and the location where females prefer to lay eggs (Reichard et al., 2009). Though territorialism has not been investigated, there will be areas within a temporal pool that have better substrate for spawning. It is unknown whether killifish have a dominance hierarchy but it is likely. Once rank is established, though individuals do not all receive the same benefits they are protected from predators and have increased access to information and resources. The formation of a dominance hierarchy among familiar males may reduce their aggression to their counterparts. This has been observed by Edenbrow and Croft (2012a), who noted that mangrove killifish (*Kryptolebias marmoratus*), showed reduced aggression and preferred to associate with familiars.

This work raises a number of potentially interesting questions for future research. For instance, future research could investigate at what age killifish show a preference for grouping and if this is linked to sexual maturity. From this experiment, I know that this social preference changes between 5 and 18 weeks old, yet it would be interesting to know the exact time point. In my previous experiment I found that juveniles show a preference to group at 7 weeks old, therefore I can narrow this change down to within a 2 week timeframe. Similarly,

I know that a preference for familiars occurs but when this preference develops is unknown. It is likely that this preference arises when males become sexually mature and dimorphic, but until more research is completed this is just speculation. This chapter has successfully uncovered African turquoise killifish's grouping preferences for the first time, yet ultimately, more research is needed to uncover the behavioural tendencies of this elusive species.

3.5. Conclusion

Adult African turquoise killifish exhibit clear social preferences; for grouping and familiar individuals, which was not revealed when testing preference for kin or when juveniles were subjected to the same tasks. This is the first time such preferences have been observed in this species. With such a short lifespan (Polacik et al., 2016), this novel system could offer unique insight into the behavioural ontogeny of sociality.

Chapter 4: General Discussion

Group living in animals arises as a consequence of the trade-offs between the costs and benefits associated with forming a group (Pitcher, 1986; Hoare et al., 2000). It is common amongst fish species yet the benefits and costs of grouping may differ. As individuals age, their life history strategies change and their tendency to join a group adjusts accordingly. Some individuals group solely as fry or juveniles (lemon sharks (*Negaprion brevirostris*) (Guttridge et al., 2009)), whilst others group solely as adults (e.g. Atlantic salmon smolts (*Salmo salar*) (Riley, 2007), bridled monocle bream (*Scolopsis bilineata*) (Radford et al., 2011) and bluehead wrasse (*Thalassoma bifasciatum*) (Searcy & Sponaugle, 2001)).

In this study, I wanted to uncover the social preferences of African turquoise killifish. I aimed to find out whether killifish had a preference for grouping, kin and familiar individuals. To my knowledge, it is the first time this has been documented in the literature. To do this, I tested the methodology and found that for my study species, a Y-maze was a more suitable test arena than the standard binary choice test. I found that juvenile African turquoise killifish showed no preference for grouping. In addition, they did not associate with kin or familiar individuals. In contrast, in adult African turquoise killifish, I observed a grouping preference. Both female and male killifish showed a propensity to group, yet when analysing preference for kin and familiars only males were found to significantly associate with familiars. This preference for familiars was modulated by the number of females in the stimulus tank; as the number decreased, preference for familiars increased, suggesting a preference for familiar males.

4.1 Developing a method to study sociability in the African turquoise killifish

Initially, I examined African turquoise killifish social preferences using the standard binary choice test most commonly used in grouping experiments. This comprises of a tank which contains 3 compartments and the fish is placed in the central one. The fish is given a choice between the two side compartments, one or both of which contain a stimuli (see Brown, 2002; Gómez-Laplaza & Gerlai, 2011; Batzina et al., 2014). I found that at 3, 5 and 7 weeks old, using the standard binary choice test arena, African turquoise killifish showed no preference to group.

Despite the standard binary choice test being a popular method for choice experiments based on the assumption that the two choices are independent, for accurate results, it requires additional information about the species in question, including information about the distance between two individuals who are considered within the same group. This is species-specific and called the inter-individual distance. African turquoise killifish inter-individual distance is unknown. It is possible that whilst using the standard binary choice tank, the fish may still consider themselves grouping in all three zones of the main compartment (social, neutral and non-social). In all three compartments, visual contact is maintained with the stimulus fish, therefore despite being in the non-social zone, a fish could still consider itself grouping if their inter-individual distance was large. It would therefore be difficult to create an accurate standard binary choice test to reflect natural grouping proximity when all choice options are simultaneously visible.

It is possible to create a standard binary choice test arena that has a choice chamber large enough so that the test fish does not perceive it is shoaling in the 'no preference' zone. This however is something I believe future researchers could investigate as it requires a lot of manipulation of test arenas and was beyond the scope of my study. I did not want to test the distance the fish perceived they were shoaling, simply whether they were shoaling or not. Now we know more about their social behaviour, this type of study would be beneficial.

To date, the inter-individual distance and the factors affecting it, are currently unknown in the African turquoise killifish. I felt, due to the lack of information on the African turquoise killifish, the binary choice test was not applicable. To be sure fish were considered to be grouping, I wanted a test in which, once an individual entered a compartment containing stimulus fish, they lost visual contact with the other compartment. Thus, decreasing the chance of inaccurate measurements through discrepancies with inter-individual distance. To do this, I used a Y-maze. Its set-up enables grouping preference to be measured in the same way as the standard binary choice tests; preference of a focal fish for a compartment over the total time spent in both compartments, yet, importantly in the Y-maze, once a fish has entered a compartment it loses visual contact with the other compartment. In contrast to the standard binary choice test results, at 7 weeks old, using the Y-maze test arena, juveniles showed a significant propensity to group.

4.2 Patterns of sociability across the lifespan

Despite grouping in fish being such a widespread and well-researched phenomenon, studies documenting group living within an entire species' lifespan are lacking. Many common laboratory species have long lifespans and due to ecological, environmental and research constraints (Hu & Brunet, 2018), their grouping preferences cannot be recorded for a prolonged period of time. For research to progress, there is a need for a laboratory species with an intrinsically short lifespan. Using the African turquoise killifish, a short-lived model organism, I could observe the changes to sociality, kin recognition and familiarity as individuals age, within a short timeframe.

From personal observations, it was apparent that as juveniles in nursery tanks, African turquoise killifish did not appear to aggregate. This was confirmed by my experiments in chapter 3, where juveniles at 5 weeks old showed no preference to group. Although it is rare in the literature, juveniles of several fish species are known to remain solitary (see Searcy & Sponaugle, 2001; Guttridge et al., 2009; Radford et al., 2011). Individuals may not have acquired the necessary sensory requirements for grouping, such as a recognition template (the ability to identify others via certain key characteristics (Mateo, 2004), or it simply may not be beneficial for them to group at an early age.

It is possible that propensity to group is linked to the age of the individual and that there are different costs and benefits of grouping between juveniles and adults. In chapter 3, fish at 5 weeks old had no preference for group living. In contrast, in chapter 2, 7 week old fish showed a propensity to group. At 7 weeks old fish

are maturing and becoming sexually dimorphic and dichromatic (Polacik et al., 2016). Their grouping tendencies at this age suggest group living may confer a reproductive advantage. Information about their short lifespan, which is constrained by their habitat, suggests that these fish rapidly mature in order to maximise reproduction and mating opportunities (Polacik et al., 2016). Grouping for mating is only a fitness advantage for those that are sexually mature and therefore should not be prevalent in juveniles (Pitcher, 1986).

Studies are beginning to emerge detailing the environmental and ecological aspects of their habitat preferences (see Reichard et al., 2014; Furness, 2016; Vrtílek, Žák, Polačik, et al., 2018; Vrtílek, Žák, Pšenička, et al., 2018 for examples), yet, their encounters with predators are speculatory. From personal observations, these fish do not show simple anti-predator responses when in their home tanks; they do not hide in refuge and are exploratory despite disturbances from observers. Additionally, though a standard protocol for laboratory feeding has been established (Polacik et al., 2016), African turquoise killifish's diet in the wild is unknown. Both predatory risk and foraging activity affect grouping in many species (e.g. sea skaters (Foster & Treherne, 1981), European minnows (Magurran, 1990) and Trinidadian guppies (Huizinga et al., 2009)), however species-specific research is required to determine how, and to what extent, these factors will affect African turquoise killifish.

In my experiments, I found that adult killifish of both sexes demonstrate a grouping preference. It is possible that adult male and female African turquoise killifish group for similar reasons to other species; guppies spend more time in large social groups when in the presence of predators (Magurran & Seghers,

1994), goldfish spend more time foraging when in larger groups (Magurran & Pitcher, 1983), whilst three-spined sticklebacks' feeding success improved in larger groups (Ranta & Kaitala, 1991). Yet it is most likely, due to their short lifespan, unpredictable habitat and unknown predation risk, that adult African turquoise killifish group to maximise mating opportunities as previously mentioned. Further work will uncover why, as adults, African turquoise killifish group, yet as juveniles they show no preference.

4.3 Impact of familiarity on grouping behaviour

To my knowledge this is the first instance of familial recognition demonstrated in African turquoise killifish. I found that when observing males, they preferentially associated with other familiar individuals, yet only when the number of females decreased. This suggests a preference for familiars when they are of the same sex. This is not uncommon in fish and has been observed in male guppies, which show a preference for familiar males (Croft et al., 2004), and female guppies, which group more cohesively in familiar same-sex groups, promoting information transfer and anti-predator defences (Davis et al., 2017). In regards to grouping, associating with familiars is advantageous in a range of contexts. It is possible that male African turquoise killifish group with other familiar males for reasons such as reduced aggression, increased group cohesion or anti-predator defences. Until more is known about the environment in which African turquoise killifish inhabit, it is impossible to accurately determine why males show a preference for other familiar males.

4.3 Impact of kinship on grouping behaviour

Generally, when a species produces a number of offspring at once, in a suitable natal habitat, it creates an environment where kin cohorts can form and recognition can take place. Kin recognition, like recognition of familiars, is beneficial in a number of ways as individuals are able to bias and participate in altruistic behaviours towards related individuals (Arnold, 2000). My experiments provide no evidence that African turquoise killifish are capable of kin recognition, despite being able to recognise familiars and being in an environment where kin are likely to interact. I theorise that due to their isolated habitat, it is likely that inbreeding will occur. Similarly, from personal observations, cannibalism is apparent. Consequently, it may be that kin recognition is not an essential requirement for optimal living and is therefore not part of their repertoire.

4.4 Avenues for future research

African turquoise killifish have tremendous potential as a system for behavioural-ontogenetic research, but challenges arose due to mortality. The results of my thesis show that juvenile and adult African turquoise killifish differ in their grouping tendencies, yet due to the mortality and changes to group size, specific comparisons could not be made. Further work is necessary to uncover when these grouping preferences change and what the causes of these changes are.

One of the key aims of my thesis was to explore the social tendencies of the African turquoise killifish, as this information is currently missing. This work involved understanding whether African turquoise killifish preferred to associate with conspecifics or remain solitary. To develop this further it would be necessary to understand the mechanisms that African turquoise killifish use to recognise

other individuals. For instance, future work can focus on the use of visual or olfactory cues to aggregate, and what benefits are accrued by grouping with familiar individuals. This section will discuss the ideas and information needed to progress this work further.

4.4.1 Understanding recognition and what cues African turquoise killifish use in social recognition

To explain why interactions between individuals are different, it is important to understand how animals recognise each other. A recognition template is at the forefront of studies investigating recognition systems (Mateo, 2004). These key characteristics are often referred to as cues and are of a sensory nature. In fish, these include visual, acoustic and olfactory cues (Partridge & Pitcher, 1980; Plenderleith et al., 2005; Spence & Smith, 2007).

In my experiments, both visual and olfactory cues were available to the focal fish. To allow this, perforated, transparent barriers were implemented. This is common in choice experiments (see Brown, 2002; Ward et al., 2007; Cattelan et al., 2017), yet to determine which are the primary cues used by individuals, we must disentangle the two and test them separately. To do this, many studies modify the barriers between the focal and stimulus fish and either have perforated, opaque barriers to allow olfactory contact only (Johannesen et al., 2012), or non-perforated, transparent barriers to allow for visual contact only (Krause & Godin, 1996).

Examples of these types of experiments implemented on fish species include female annual killifish (*Austrolebias reicherti*), which preferred to associate with males based solely on olfactory cues, and were not able to discriminate using visual cues alone (Reyes Blengini et al., 2018), female thicklip pupfish (*Cyprinodon labiosus*), which can discriminate between male conspecifics and heterospecifics using olfactory cues, but not visual cues (Kodric-Brown & Strecker, 2008), and female rainbowfish (*Melanotaenia eachamensis*), which could identify relatives using visual and olfactory cues together, but could not determine relatedness using olfactory cues alone (Arnold, 2000). Implemented on African turquoise killifish, this could provide us with information on whether they use visual, olfactory or a combination of cues to recognise conspecifics.

4.4.2 Increase research on familiarity and whether the benefits apply to these individuals

Recognition at a basic level – the ability to discriminate between conspecifics and heterospecifics – is beneficial for many species (those that form swarms, herds and flocks (Krause & Ruxton, 2002)). Yet, for most, a more sophisticated level of recognition is necessary. In particular, the recognition of familiar and related conspecifics allows individuals to modify their behaviour accordingly. Given the benefits accrued from grouping with familiar individuals (greater group cohesion (Pitcher, 1986; Fitzgerald & Morrissette, 1992; Chivers et al., 1995; Jordan et al., 2010), decreased aggression towards other group members (Utne-Palm & Hart 2003) and increased exploratory behaviour (Bhat & Magurran, 2006)) it would be interesting to study if these are apparent in the male African turquoise killifish groups.

To investigate this, previous studies have manipulated laboratory populations of fish. Chivers et al. (1995) added predatory odours to a tank and influenced grouping proximity. This study found that greater group cohesion was achieved in groups of familiar fish. Similarly, Utne-Palm and Hart (2003), observed aggressive tendencies, such as chasing, biting and retreating behaviours, between two or more individuals at a food patch. They found that groups of familiar fish decreased their aggressive interactions towards one another. Finally, Bhat and Magurran (2006), tested exploratory behaviour and emergence from a shelter in both familiar and unfamiliar fish and found increased exploratory behaviour in familiar groups. With this in mind, if I were to undertake these tests on familiar and unfamiliar groups of male African turquoise killifish, I would expect to see decreased aggression, increased group cohesion and increased exploratory behaviour in familiar groups and the opposite effect in groups of unfamiliar males.

4.4.3 Behavioural ontogeny

Sociality and aging are closely linked in animals. Through sociality and grouping, an individual's fitness, longevity and mortality can be affected and affect the fitness of others (Bourke, 2007). Research on the connections between sociality and aging are lacking and, in vertebrate research, are usually impeded by the long lifespan of the model organisms. The African turquoise killifish could provide a model system, with a short lifespan, that can test the implications of sociality on aging.

4.5 Conclusion

The findings from my thesis are the first to provide basic information on the social behaviour of African turquoise killifish. Group living is a prominent aspect of many species' lives and has both benefits and costs associated with it. I aimed to explore the social behaviour of the African turquoise killifish through investigating their grouping tendencies with conspecifics.

I found that the standard binary choice test was not an appropriate method for my study species so I used a Y-maze as a substitute, which proved to be a more accurate experimental design. Using the Y-maze I found that as 5 week old juveniles, African turquoise killifish have no grouping tendencies, yet at 7 weeks old they do. Furthermore adults, both males and females prefer to associate with conspecifics.

Finally, I explored whether African turquoise killifish are capable of recognising related and familiar individuals. In addition to their lack of preference for grouping, 5 week old juveniles also had no preference for kin or familiars. Similarly, female African turquoise killifish also showed no preference for kin or familiars. In contrast, male African turquoise killifish were found to preferentially associate with familiars. This preference was only apparent when modulated by the number of females in the stimulus group. As the number of females decreased, preference for familiars increased, suggesting a preference for familiar males.

Further research should investigate whether grouping preference is determined by olfactory or visual cues and whether familiarity confers similar benefits in African turquoise killifish as it does in other species. Increased knowledge on this

species will provide useful insight into the behaviour and ecology of both this organism and other short-lived species and into the development of social behaviour more generally. This thesis has attempted to identify the grouping tendencies of this elusive species and ultimately increase the knowledge on what is becoming an emerging model organism in scientific research.

References

Akaike, H. (1973). Information theory and an extension of the maximum likelihood principle. In E. Parzen, K. Tanabe, & G. Kitagawa (Eds.), *Selected Papers of Hirotugu Akaike* (pp. 199-213). New York, NY: Springer New York.

Alados, C., & Escós, J. (1992). The determinants of social status and the effect of female rank on reproductive success in Dama and Cuvier's gazelles. *Ethology, Ecology and Evolution, 4*, 151-164. doi:10.1080/08927014.1992.9525336

Arnold, K. (2000). Kin recognition in rainbowfish (Melanotaenia eachamensis): sex, sibs and shoaling. *Behavioral Ecology and Sociobiology, 48*(5), 385-391.

Aubin, T., & Jouventin, P. (1998). Cocktail-party effect in king penguin colonies. *Proceedings of the Royal Society B: Biological Sciences, 265*(1406), 1665-1673. doi:10.1098/rspb.1998.0486

Baker, P. J., Funk, S. M., Bruford, M. W., & Harris, S. (2004). Polygynandry in a red fox population: implications for the evolution of group living in canids? *Behavioral Ecology, 15*(5), 766-778. doi:10.1093/beheco/arh077

Balcombe, J. P. (1990). Vocal recognition of pups by mother Mexican free-tailed bats, Tadarida brasiliensis mexicana. *Animal Behaviour, 39*(5), 960-966. doi:https://doi.org/10.1016/S0003-3472(05)80961-3

Barber, I., Hoare, D., & Krause, J. (2000). Effects of parasites on fish behaviour: a review and evolutionary perspective. *Reviews in Fish Biology and Fisheries, 10*(2), 131-165. doi:10.1023/A:1016658224470

Barber, I., & Wright, H. A. (2001). How strong are familiarity preferences in shoaling fish? *Animal Behaviour, 61*(5), 975-979. doi:https://doi.org/10.1006/anbe.2000.1665

Barnett, C. (1982). The chemosensory responses of young cichlid fish to parents and predators. *Animal Behaviour, 30*(1), 35-42. doi:https://doi.org/10.1016/S0003-3472(82)80234-0

Barton, K. (2018). Multi-Model Inference (Version 1.42.1) [Package].

Batzina, A., Sotirakoglou, K., & Karakatsouli, N. (2014). The preference of 0+ and 2+ gilthead seabream Sparus aurata for coloured substrates or no-substrate. *Applied Animal Behaviour Science, 151*, 110-116. doi:https://doi.org/10.1016/j.applanim.2013.11.013

Behrmann-Godel, J., Gerlach, G., & Eckmann, R. (2006). Kin and population recognition in sympatric Lake Constance perch (Perca fluviatilis L.): can assortative shoaling drive population divergence? *Behavioral Ecology and Sociobiology, 59*(4), 461-468. doi:10.1007/s00265-005-0070-3

Bernstein, I. S. (1976). Dominance, aggression and reproduction in primate societies. *Journal of Theoretical Biology, 60*(2), 459-472. doi:https://doi.org/10.1016/0022-5193(76)90072-2

Bhat, A., & Magurran, A. E. (2006). Benefits of familiarity persist after prolonged isolation in guppies. *Journal of Fish Biology, 68*(3), 759-766. doi:10.1111/j.0022-1112.2006.00963.x

Binoy, V., Kasturirangan, R., & Sinha, A. (2015). Sensory cues employed for the acquisition of familiarity-dependent recognition of a shoal of conspecifics by climbing perch (Anabas testudineus Bloch). *Journal of biosciences, 40*(2), 225-232.

Bleckmann, H. (1986). Role of the lateral line in fish behaviour. In *The behaviour of teleost fishes* (pp. 177-202): Springer.

Bourke, A. F. G. (2007). Kin Selection and the Evolutionary Theory of Aging. *Annual Review of Ecology, Evolution, and Systematics, 38*(1), 103-128. doi:10.1146/annurev.ecolsys.38.091206.095528

Bowers, J. M., & Alexander, B. K. (1967). Mice: Individual recognition by olfactory cues. *Science, 158*(3805), 1208.

Brooks, M., Kristensen, K., J., V. B. K., Magnusson, A., Berg, C. W., Nielson, A., . . . Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal., 9*(2), 378-400. doi:https://doi.org/10.1101/132753

Brown, C. (2002). Do female rainbowfish (Melanotaenia spp.) prefer to shoal with familiar individuals under predation pressure? *Journal of Ethology, 20*(2), 89-94. doi:10.1007/s10164-002-0059-6

Brown, C. R., & Brown, M. B. (2004). Empirical measurement of parasite transmission between groups in a colonial bird. *Ecology, 85*(6), 1619-1626. doi:10.1890/03-0206

Brown, G. E., & Brown, J. A. (1996). Does kin-biased territorial behavior increase kin-biased foraging in juvenile salmonids? *Behavioral Ecology, 7*(1), 24-29. doi:10.1093/beheco/7.1.24

Buckley, F. G., & Buckley, P. A. (1972). THE BREEDING ECOLOGY OF ROYAL TERNS STERNA (THALASSEUS) MAXIMA MAXIMA. *Ibis, 114*(3), 344-359. doi:10.1111/j.1474-919X.1972.tb00832.x

Budaev, S. V. (1997). Alternative styles in the European wrasse, Symphodus ocellatus: boldness-related schooling tendency. *Environmental Biology of Fishes, 49*(1), 71-78. doi:10.1023/a:1007380212475

Burnham, K. P., & Anderson, D. R. (2003). *Model selection and multimodel inference: a practical information-theoretic approach*: Springer Science & Business Media.

Buske, C., & Gerlai, R. (2011). Shoaling develops with age in Zebrafish (Danio rerio). *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 35*(6), 1409-1415. doi:https://doi.org/10.1016/j.pnpbp.2010.09.003

Butler, D. (2009). asreml: asreml () fits the linear mixed model. (Version R package version 3). Retrieved from www.vsni.co.uk

Cattelan, S., Lucon-Xiccato, T., Pilastro, A., & Griggio, M. (2017). Is the mirror test a valid measure of fish sociability? *Animal Behaviour, 127*, 109-116.

Cellerino, A., Valenzano, D. R., & Reichard, M. (2015). From the bush to the bench: the annual Nothobranchius fishes as a new model system in biology. *Biological Reviews, 91*(2), 511-533. doi:10.1111/brv.12183

Chapple, D. G. (2003). Ecology, life-history, and behavior in the australian scincid genus egernia, with comments on the evolution of complex sociality in lizards. *Herpetological Monographs, 17*, 145-180.

Cheng, J., Sun, Y., & Ji, L. (2010). A call-independent and automatic acoustic system for the individual recognition of animals: A novel model using four passerines. *Pattern Recognition, 43*(11), 3846-3852. doi:https://doi.org/10.1016/j.patcog.2010.04.026

Chivers, D. P., Brown, G. E., & Smith, R. J. F. (1995). Familiarity and shoal cohesion in fathead minnows (Pimephales promelas): implications for antipredator behaviour. *Canadian Journal of Zoology, 73*(5), 955-960. doi:10.1139/z95-111

Coffin, H. R., Watters, J. V., & Mateo, J. M. (2011). Odor-based recognition of familiar and related conspecifics: A first test conducted on captive Humboldt Penguins (Spheniscus humboldti). *PLoS One, 6*(9), e25002. doi:10.1371/journal.pone.0025002

Cooper, S. M. (1991). Optimal hunting group size: the need for lions to defend their kills against loss to spotted hyaenas. *African Journal of Ecology, 29*(2), 130-136. doi:doi:10.1111/j.1365-2028.1991.tb00993.x

Coster-Longman, C., Landi, M., & Turillazzi, S. (2002). The role of passive defense (selfish herd and dilution effect) in the gregarious nesting of Liostenogaster Wasps (Vespidae, Hymenoptera, Stenogastrinae). *Journal of Insect Behavior, 15*(3), 331-350. doi:10.1023/a:1016213125161

Côté, I. M., & Poulinb, R. (1995). Parasitism and group size in social animals: a meta-analysis. *Behavioral Ecology, 6*(2), 159-165. doi:10.1093/beheco/6.2.159

Cribari-Neto, F., & Zeileis, A. (2010). Beta Regression in R. *Journal of Statisical Software, 34*(2), 1-24.

Croft, D. P., Arrowsmith, B. J., Bielby, J., Skinner, K., White, E., Couzin, I. D., . . . Krause, J. (2003). Mechanisms underlying shoal composition in the Trinidadian guppy, Poecilia reticulata. *Oikos, 100*(3), 429-438. doi:10.1034/j.1600-0706.2003.12023.x

Croft, D. P., Arrowsmith, B. J., Webster, M., & Krause, J. (2004). Intra-sexual preferences for familiar fish in male guppies. *Journal of Fish Biology, 64*(1), 279-283. doi:10.1111/j.1095-8649.2004.00305.x

Davis, S., Lukeman, R., Schaerf, T. M., & Ward, A. J. W. (2017). Familiarity affects collective motion in shoals of guppies (Poecilia reticulata). *Royal Society Open Science, 4*(9), 170312. doi:10.1098/rsos.170312

Dolan, T. W., & Butler, M. J. (2006). The adaptive value of aggregation among juvenile Caribbean spiny lobster: an evaluation using individual-based modeling. *Journal of Crustacean Biology, 26*(4), 565-578.

Edeline, E., Dufour, S., & Elie, P. (2009). Proximate and ultimate control of eel continental dispersal. In G. Van den Thillart, S. Dufour, & J. C. Rankin (Eds.), *Spawning migration of the European eel* (Vol. 30, pp. 433-461). Netherlands: Springer.

Edenbrow, M., & Croft, D. P. (2011). Behavioural types and life history strategies during ontogeny in the mangrove killifish, Kryptolebias marmoratus. *Animal Behaviour, 82*(4), 731-741. doi:https://doi.org/10.1016/j.anbehav.2011.07.003

Edenbrow, M., & Croft, D. P. (2012a). Kin and familiarity influence association preferences and aggression in the mangrove killifish Kryptolebias marmoratus. *Journal of Fish Biology, 80*(3), 503-518. doi:10.1111/j.1095-8649.2011.03181.x

Edenbrow, M., & Croft, D. P. (2012b). Kin and familiarity influence association preferences and aggression in the mangrove killifish Kryptolebias marmoratus. *Journal of Fish Biology, 80*(3), 503-518. doi:10.1111/j.1095-8649.2011.03181.x

Edenbrow, M., & Croft, D. P. (2013). Environmental and genetic effects shape the development of personality traits in the mangrove killifish Kryptolebias marmoratus. *Oikos, 122*(5), 667-681. doi:10.1111/j.1600-0706.2012.20556.x

Ellison, A., Leaniz Carlos, G., & Consuegra, S. (2013). Inbred and furious: negative association between aggression and genetic diversity in highly inbred fish. *Molecular Ecology, 22*(8), 2292-2300. doi:10.1111/mec.12238

Engeszer, R. E., Da Barbiano, L. A., Ryan, M. J., & Parichy, D. M. (2007). Timing and plasticity of shoaling behaviour in the zebrafish, Danio rerio. *Animal Behaviour, 74*(5), 1269-1275.

Engström-Öst, J., Karjalainen, M., & Viitasalo, M. (2006). Feeding and Refuge Use by Small Fish in the Presence of Cyanobacteria Blooms. *Environmental Biology of Fishes, 76*(1), 109-117. doi:10.1007/s10641-006-9013-8

Evans, K. E., & Harris, S. (2008). Adolescence in male African elephants, Loxodonta africana, and the importance of sociality. *Animal Behaviour, 76*(3), 779-787. doi:https://doi.org/10.1016/j.anbehav.2008.03.019

Ewulonu, U. K., Haas, R., & Turner, B. J. (1985). A multiple sex chromosome system in the annual killifish, Nothobranchius guentheri. *Copeia, 1985*(2), 503-508. doi:10.2307/1444868

Faucher, K., Parmentier, E., Becco, C., Vandewalle, N., & Vandewalle, P. (2010). Fish lateral system is required for accurate control of shoaling behaviour. *Animal Behaviour, 79*(3), 679-687. doi:https://doi.org/10.1016/j.anbehav.2009.12.020

Fitzgerald, G. J., & Morrissette, J. (1992). Kin recognition and choice of shoal mates by threespine sticklebacks. *Ethology Ecology & Evolution, 4*(3), 273-283.

Ford, M. I., Elvidge, C. K., Baker, D., Pratt, T. C., Smokorowski, K. E., Sills, M., . . . Cooke, S. J. (2018). Preferences of age-0 white sturgeon for different colours and strobe rates of LED lights may inform behavioural guidance strategies. *Environmental Biology of Fishes, 101*(4), 667-674. doi:10.1007/s10641-018-0727-1

Foster, W. A., & Treherne, J. E. (1981). Evidence for the dilution effect in the selfish herd from fish predation on a marine insect. *Nature, 293*, 466-467. doi:10.1038/293466a0

Froese, R., & Pauly, D. (2017). FishBase. 06/2017. Retrieved from http://www.fishbase.org/search.php

Frommen, J. G., & Bakker, T. C. (2004). Adult three-spined sticklebacks prefer to shoal with familiar kin. *Behaviour, 141*(11), 1401-1409.

Frommen, J. G., & Bakker, T. C. M. (2006). Inbreeding avoidance through non-random mating in sticklebacks. *Biology Letters, 2*(2), 232.

Frommen, J. G., Luz, C., Mazzi, D., & Bakker, T. C. M. (2008). Inbreeding depression affects fertilization success and survival but not breeding coloration in threespine sticklebacks. *Behaviour, 145*(4/5), 425-441.

Frommen, J. G., Mehlis, M., Brendler, C., & Bakker, T. C. M. (2007). Shoaling decisions in three-spined sticklebacks (Gasterosteus aculeatus): Familiarity, kinship and inbreeding. *Behavioral Ecology and Sociobiology, 61*(4), 533-539.

Furness, A. (2016). The evolution of an annual life cycle in killifish: adaptation to ephemeral aquatic environments through embryonic diapause. *Biological Reviews, 91*(3), 796-812. doi:10.1111/brv.12194

Gamboa, G. J., Reeve, H. K., & Pfennig, D. W. (1986). The evolution and ontogeny of nestmate recognition in social wasps. *Annual review of entomology, 31*(1), 431-454.

Gazda, S. K., Connor, R. C., Edgar, R. K., & Cox, F. (2005). A division of labour with role specialization in group–hunting bottlenose dolphins (Tursiops truncatus) off Cedar Key, Florida. *Proceedings of the Royal Society of London B: Biological Sciences, 272*(1559), 135-140.

Gelman, A. (2008). Scaling regression inputs by dividing by two standard deviations. *Statistics in medicine, 27*(15), 2865-2873.

Gereta, E., Ole Meing'ataki, G. E., Mduma, S., & Wolanski, E. (2004). The role of wetlands in wildlife migration in the Tarangire ecosystem, Tanzania. *Wetlands Ecology and Management, 12*(4), 285-299. doi:10.1007/s11273-005-3499-2

Gerlach, G., Hodgins-Davis, A., Avolio, C., & Schunter, C. (2008). Kin recognition in zebrafish: a 24-hour window for olfactory imprinting. *Proceedings of the Royal Society B: Biological Sciences, 275*(1647), 2165-2170.

Gerlach, G., & Lysiak, N. (2006). Kin recognition and inbreeding avoidance in zebrafish, Danio rerio, is based on phenotype matching. *Animal Behaviour, 71*, 1371-1377. doi:10.1016/j.anbehav.2005.10.010

Gómez-Laplaza, L. M. (2006). Shoal choice in juvenile angelfish (Pterophyllum scalare): effects of social status and activity. *Ethology Ecology & Evolution, 18*(4), 261-273. doi:10.1080/08927014.2006.9522695

Gómez-Laplaza, L. M., & Gerlai, R. (2011). Spontaneous discrimination of small quantities: shoaling preferences in angelfish (Pterophyllum scalare). *Animal Cognition, 14*(4), 565-574. doi:10.1007/s10071-011-0392-7

Griffiths, S. W. (2003). Learned recognition of conspecifics by fishes. *Fish and Fisheries, 4*(3), 256-268. doi:10.1046/j.1467-2979.2003.00129.x

Griffiths, S. W., Brockmark, S., Hojesjo, J., & Johnsson, J. I. (2004). Coping with divided attention: the advantage of familiarity. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 271*(1540), 695-699.

Griffiths, S. W., & Magurran, A. E. (1997). Schooling preferences for familiar fish vary with group size in a wild guppy population. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 264*(1381), 547-551.

Griffiths, S. W., & Magurran, A. E. (1999). Schooling decisions in guppies are based on familiarity rather than kin recognition by phenotype matching. *Behavioral Ecology and Sociobiology, 45*, 437-443.

Grueber, C. E., Nakagawa, S., Laws, R. J., & Jamieson, I. G. (2011). Multimodel inference in ecology and evolution: challenges and solutions. *Journal of Evolutionary Biology, 24*(4), 699-711. doi:10.1111/j.1420-9101.2010.02210.x

Guttridge, T. L., Gruber, S. H., Gledhill, K. S., Croft, D. P., Sims, D. W., & Krause, J. (2009). Social preferences of juvenile lemon sharks, Negaprion brevirostris. *Animal Behaviour, 78*(2), 543-548. doi:https://doi.org/10.1016/j.anbehav.2009.06.009

Haas, R. (1976). Sexual selection in Nothobranchius guentheri (Pisces: cyprinodontidae). *Evolution, 30*(3), 614-622.

Harel, I., Valenzano, D. R., & Brunet, A. (2016). Efficient genome engineering approaches for the short-lived African turquoise killifish. *Nature Protocols., 11*, 2010-2028. doi:doi:10.1038/nprot.2016.103

Hass, C. C., & Valenzuela, D. (2002). Anti-predator benefits of group living in white-nosed coatis (Nasua narica). *Behavioral Ecology and Sociobiology, 51*(6), 570-578. doi:10.1007/s00265-002-0463-5

Hay, D. E., & McKinnell, S. M. (2002). Tagging along: association among individual Pacific herring (Clupea pallasi) revealed by tagging. *Canadian Journal of Fisheries and Aquatic Sciences, 59*(12), 1960-1968. doi:10.1139/f02-141

Hebblewhite, M., & Pletscher, D. H. (2002). Effects of elk group size on predation by wolves. *Canadian Journal of Zoology, 80*(5), 800-809.

Hoare, D., Krause, J., Peuhkuri, N., & Godin, J. G. (2000). Body size and shoaling in fish. *Journal of Fish Biology, 57*(6), 1351-1366.

Hoare, D. J., Couzin, I. D., Godin, J.-G., & Krause, J. (2004). Context-dependent group size choice in fish. *Animal Behaviour, 67*(1), 155-164.

Höjesjö, J., Johnsson, J. I., Petersson, E., & Järvi, T. (1998). The importance of being familiar: individual recognition and social behavior in sea trout (Salmo trutta). *Behavioral Ecology, 9*(5), 445-451. doi:10.1093/beheco/9.5.445

Hu, C.-K., & Brunet, A. (2018). The African turquoise killifish: A research organism to study vertebrate aging and diapause. *Aging Cell, 17*(3), e12757. doi:10.1111/acel.12757

Huizinga, M., Ghalambor C, K., & Reznick D, N. (2009). The genetic and environmental basis of adaptive differences in shoaling behaviour among populations of Trinidadian guppies, Poecilia reticulata. *Journal of Evolutionary Biology, 22*(9), 1860-1866. doi:10.1111/j.1420-9101.2009.01799.x

Ioannou, C., Bartumeus, F., Krause, J., & Ruxton, D. (2011). Unified effects of aggregation reveal larger prey groups take longer to find. *Proceedings of the Royal Society B: Biological Sciences, 278*(1720), 2985-2990. doi:10.1098/rspb.2011.0003

Jarvis, J. U. M. (1981). Eusociality in a mammal: cooperative breeding in naked mole-rat colonies. *Science, 212*(4494), 571-573.

Johannesen, A., Dunn, A. M., & Morrell, L. J. (2012). Olfactory cue use by three-spined sticklebacks foraging in turbid water: prey detection or prey location? *Animal Behaviour, 84*(1), 151-158. doi:https://doi.org/10.1016/j.anbehav.2012.04.024

Johnson, M. B., Lafferty, K. D., van Oosterhout, C., & Cable, J. (2011). Parasite transmission in social interacting hosts: Monogenean epidemics in guppies. *PLoS One, 6*(8), e22634. doi:10.1371/journal.pone.0022634

Jones, K. A., Croft, D. P., Ramnarine, I. W., & Godin, J. G. J. (2010). Size-Assortative Shoaling in the Guppy (Poecilia reticulata): The Role of Active Choice. *Ethology, 116*(2), 147-154. doi:doi:10.1111/j.1439-0310.2009.01727.x

Jordan, L. A., Wong, M. Y. L., & Balshine, S. S. (2010). The effects of familiarity and social hierarchy on group membership decisions in a social fish. *Biology Letters, 6*(3), 301-303.

Jubb, R. A. (1971). A New *Nothobranchius* (Pisces Cyprinodontidae) from South-eastern Rhodesia. *Journal of the American Killifish Association, 8*(1), 12-19.

Judd, T. M., & Sherman, P. W. (1996). Naked mole-rats recruit colony mates to food sources. *Animal Behaviour, 52*(5), 957-969. doi:https://doi.org/10.1006/anbe.1996.0244

Kim, Y., Nam, H. G., & Valenzano, D. R. (2016). The short-lived African turquoise killifish: an emerging experimental model for ageing. *Disease models & mechanisms, 9*(2), 115-129.

Kodric-Brown, A., & Strecker, U. (2008). Responses of Cyprinodon maya and C. labiosus females to visual and olfactory cues of conspecific and heterospecific males. *Biological Journal of the Linnean Society, 74*(4), 541-548. doi:10.1111/j.1095-8312.2001.tb01411.x

Komdeur, J. (1992). Importance of habitat saturation and territory quality for evolution of cooperative breeding in the Seychelles warbler. *Nature, 358*, 493. doi:10.1038/358493a0

König, B. (1997). Cooperative care of young in mammals. *Naturwissenschaften, 84*(3), 95-104.

Krause, E. T., Krüger, O., Kohlmeier, P., & Caspers, B. A. (2012). Olfactory kin recognition in a songbird. *Biology Letters, 8*(3), 327-329.

Krause, J. (1993). The influence of hunger on shoal size choice by three-spined sticklebacks, Gasterosteus aculeatus. *Journal of Fish Biology, 43*(5), 775-780. doi:10.1111/j.1095-8649.1993.tb01154.x

Krause, J. (1994). The influence of food competition and predation risk on size-assortative shoaling in juvenile chub (Leuciscus cephalus). *Ethology, 96*(2), 105-116. doi:10.1111/j.1439-0310.1994.tb00886.x

Krause, J., Butlin, R. K., Peuhkuri, N., & Pritchard, V. L. (2007). The social organization of fish shoals: a test of the predictive power of laboratory experiments for the field. *Biological Reviews, 75*(4), 477-501. doi:10.1111/j.1469-185X.2000.tb00052.x

Krause, J., & Godin, J.-G. J. (1996). Influence of parasitism on shoal choice in the Banded Killifish (Fundulus diaphanus, Teleostei, Cyprinodontidae). *Ethology, 102*(1), 40-49. doi:10.1111/j.1439-0310.1996.tb01102.x

Krause, J., & Godin, J. G. J. (1994). Shoal choice in the banded killifish (Fundulus diaphanus, Teleostei, Cyprinodontidae): effects of predation risk, fish size, species composition and size of shoals. *Ethology, 98*(2), 128-136.

Krause, J., Hoare, D. J., Croft, D., Lawrence, J., Ward, A., Ruxton, G. D., . . . James, R. (2000). Fish shoal composition: mechanisms and constraints. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 267*(1456), 2011.

Krause, J., & Ruxton, G. (2002). *Living in groups*. New York: Oxford University Press.

Lachlan, R. F., Crooks, L., & Laland, K. N. (1998). Who follows whom? Shoaling preferences and social learning of foraging information in guppies. *Animal Behaviour, 56*(1), 181-190. doi:https://doi.org/10.1006/anbe.1998.0760

Landeau, L., & Terborgh, J. (1986). Oddity and the 'confusion effect' in predation. *Animal Behaviour, 34*(5), 1372-1380. doi:https://doi.org/10.1016/S0003-3472(86)80208-1

Ledesma, J. M., & McRobert, S. P. (2008). Innate and learned shoaling preferences based on body coloration in juvenile mollies, Poecilia latipinna. *Ethology, 114*(11), 1044-1048. doi:10.1111/j.1439-0310.2008.01560.x

Lindström, K., & Ranta, E. (1993). Social preferences by male guppies, Poecilia reticulata, based on shoal size and. *Animal Behaviour, 46*, 1029-1031.

Madsen, T., Stille, B., & Shine, R. (1996). Inbreeding depression in an isolated population of Adders Vipera berus. *Biological Conservation, 75*, 113-118. doi:10.1016/0006-3207(95)00067-4

Magnhagen, C. (2008). *Fish behaviour*: CRC Press.

Magurran, A. E. (1986). The development of shoaling behaviour in the European minnow, Phoxinus phoxinus. *Journal of Fish Biology, 29*(sA), 159-169. doi:10.1111/j.1095-8649.1986.tb05007.x

Magurran, A. E. (1990). The inheritance and development of minnow anti-predator behaviour. *Animal Behaviour, 39*(5), 834-842. doi:https://doi.org/10.1016/S0003-3472(05)80947-9

Magurran, A. E., & Pitcher, T. J. (1983). Foraging, timidity and shoal size in minnows and goldfish. *Behavioral Ecology and Sociobiology, 12*(2), 147-152. doi:10.1007/BF00343206

Magurran, A. E., & Seghers, B. H. (1994). Predator inspection behaviour covaries with schooling tendency amongst wild guppy, Poecilia Reticulata, populations in Trinidad. *Behaviour, 128*(1), 121-134. doi:doi:https://doi.org/10.1163/156853994X00073

Major, P. F. (1978). Predator-prey interactions in two schooling fishes, Caranx ignobilis and Stolephorus purpureus. *Animal Behaviour, 26*, 760-777. doi:https://doi.org/10.1016/0003-3472(78)90142-2

Masuda, R., Shoji, J. U. N., Nakayama, S., & Tanaka, M. (2003). Development of schooling behavior in Spanish mackerel (Scomberomorus niphonius) during early ontogeny. *Fisheries science, 69*(4), 772-776. doi:10.1046/j.1444-2906.2003.00685.x

Mateo, J. M. (2004). *Recognition systems and biological organization: the perception component of social recognition.* Paper presented at the Annales Zoologici Fennici.

Matyjasiak, P. (2005). Birds associate species-specific acoustic and visual cues: recognition of heterospecific rivals by male blackcaps. *Behavioral Ecology, 16*(2), 467-471. doi:10.1093/beheco/ari012

McRobert, S. P., & Bradner, J. (1998). The influence of body coloration on shoaling preferences in fish. *Animal Behaviour, 56*(3), 611-615. doi:https://doi.org/10.1006/anbe.1998.0846

Milinski, M., Griffiths, S., Wegner, K. M., Reusch, T. B. H., Haas-Assenbaum, A., & Boehm, T. (2005). Mate choice decisions of stickleback females predictably modified by MHC peptide ligands. *Proceedings of the National Academy of Sciences of the United States of America, 102*(12), 4414-4418. doi:10.1073/pnas.0408264102

Milinski, M., Pfluger, D., Külling, D., & Kettler, R. (1990). Do sticklebacks cooperate repeatedly in reciprocal pairs? *Behavioral Ecology and Sociobiology, 27*(1), 17-21. doi:10.1007/BF00183308

Miller, N. Y., & Gerlai, R. (2008). Oscillations in shoal cohesion in zebrafish (Danio rerio). *Behavioural Brain Research, 193*(1), 148-151. doi:https://doi.org/10.1016/j.bbr.2008.05.004

Miller, N. Y., & Gerlai, R. (2011). Shoaling in zebrafish: what we don't know. *Rev Neurosci, 22*(1), 17-25. doi:10.1515/rns.2011.004

Mills, D. S., & Marchant-Forde, J. N. (2010). *The encyclopedia of applied animal behaviour and welfare* (Vol. 24). Wallingford: CAB International.

Molvar, E. M., & Bowyer, R. T. (1994). Costs and benefits of group living in a recently social ungulate: The Alaskan moose. *Journal of Mammalogy, 75*(3), 621-630. doi:10.2307/1382509

Monaghan, P., & Metcalfe, N. B. (1985). Group foraging in wild brown hares: effects of resource distribution and social status. *Animal Behaviour, 33*(3), 993-999. doi:https://doi.org/10.1016/S0003-3472(85)80033-6

Morgan, M., & Godin, J. G. J. (1985). Antipredator benefits of schooling behaviour in a cyprinodontid fish, the banded killifish (Fundulus diaphanus). *Zeitschrift für Tierpsychologie, 70*(3), 236-246. doi:10.1111/j.1439-0310.1985.tb00515.x

Morgan, M. J. (1988). The effect of hunger, shoal size and the presence of a predator on shoal cohesiveness in bluntnose minnows, Pimephales notatus Rafinesque. *Journal of Fish Biology, 32*(6), 963-971. doi:10.1111/j.1095-8649.1988.tb05439.x

Morrell, L. J., Hunt, K. L., Croft, D. P., & Krause, J. (2007). Diet, familiarity and shoaling decisions in guppies. *Animal Behaviour, 74*(2), 311-319. doi:https://doi.org/10.1016/j.anbehav.2006.10.021

Nakagawa, S., & Freckleton, R. P. (2011). Model averaging, missing data and multiple imputation: a case study for behavioural ecology. *Behavioral Ecology and Sociobiology, 65*(1), 103-116.

Natt, M., Lönnstedt, O. M., & McCormick, M. I. (2017). Coral reef fish predator maintains olfactory acuity in degraded coral habitats. *PLoS One, 12*(6), e0179300. doi:10.1371/journal.pone.0179300

Neff, B. D., & Sherman, P. W. (2003). Nestling recognition via direct cues by parental male bluegill sunfish (Lepomis macrochirus). *Animal Cognition, 6*(2), 87-92. doi:10.1007/s10071-003-0166-y

Olsen, K. H., Grahn, M., Lohm, J., & Langefors, Å. (1998). MHC and kin discrimination in juvenile Arctic charr, Salvelinus alpinus (L.). *Animal Behaviour, 56*(2), 319-327. doi:https://doi.org/10.1006/anbe.1998.0837

Olsén, K. H., & JäUrvi, T. (2005). Effects of kinship on aggression and RNA content in juvenile Arctic charr. *Journal of Fish Biology, 51*(2), 422-435. doi:10.1111/j.1095-8649.1997.tb01676.x

Pangle, W. M., & Holekamp, K. E. (2010). Functions of vigilance behaviour in a social carnivore, the spotted hyaena, Crocuta crocuta. *Animal Behaviour, 80*(2), 257-267. doi:10.1016/j.anbehav.2010.04.026

Partridge, B. L. (1982). The Structure and Function of Fish Schools. *Scientific American, 246*(6), 114-123.

Partridge, B. L., & Pitcher, T. J. (1980). The sensory basis of fish schools: Relative roles of lateral line and vision. *Journal of comparative physiology, 135*(4), 315-325. doi:10.1007/bf00657647

Pavlov, D., & Kasumyan, A. (2000). Patterns and mechanisms of schooling behavior in fish: A review. *Journal of Ichthyology, 40*, 163–231.

Paxton, C. G. M. (1996). Isolation and the development of shoaling in two populations of the guppy. *Journal of Fish Biology, 49*(3), 514-520. doi:10.1111/j.1095-8649.1996.tb00046.x

Peichel, C. L. (2004). Social behavior: how do fish find their shoal mate? *Current Biology, 14*(13), 503-504. doi:https://doi.org/10.1016/j.cub.2004.06.037

Penn, D. J., & Potts, W. K. (1999). The Evolution of Mating Preferences and Major Histocompatibility Complex Genes. *The American Naturalist, 153*(2), 145-164. doi:10.1086/303166

Pérez Pérez, A., & Veà, J. J. (2000). Allogrooming behavior in Cercocebus torquatus: The case for the hygienic functional hypothesis. *Primates, 41*(2), 199-207. doi:10.1007/BF02557801

Pitcher, T., Magurran, A., & Allan, J. R. (1983). Shifts of behaviour with shoal size in Cyprinids. *Proceedings of the British freshwater Fisheries Conference, 3*, 220-228.

Pitcher, T. J. (1986). Functions of shoaling behaviour in teleosts. In T. J. Pitcher (Ed.), *Behaviour of Teleost Fishes.* London: Chapman and Hall.

Pitcher, T. J. (1989). *The Behaviour of Teleost Fishes* (Vol. 74): Wiley-Blackwell.

Pitcher, T. J., Magurran, A. E., & Edwards, J. I. (1985). Schooling mackerel and herring choose neighbours of similar size. *Marine Biology, 86*(3), 319-322. doi:10.1007/BF00397518

Pitcher, T. J., Magurran, A. E., & Winfield, I. J. (1982). Fish in larger shoals find food faster. *Behavioral Ecology and Sociobiology, 10*(2), 149-151. doi:10.1007/bf00300175

Pitcher, T. J., Partridge, B. L., & Wardle, C. S. (1976). A blind fish can school. *Science, 194*(4268), 963.

Plenderleith, M., Oosterhout, C. v., Robinson, R. L., & Turner, G. F. (2005). Female preference for conspecific males based on olfactory cues in a Lake Malawi cichlid fish. *Biology Letters, 1*(4), 411.

Polacik, M., Blazek, R., & Reichard, M. (2016). Laboratory breeding of the short-lived annual killifish Nothobranchius furzeri. *Nature Protocols., 11*(8), 1396-1413. doi:10.1038/nprot.2016.080

Polačik, M., & Janáč, M. (2017). Costly defense in a fluctuating environment - sensitivity of annual Nothobranchius fishes to predator kairomones. *Ecology and Evolution, 7*(12), 4289-4298. doi:10.1002/ece3.3019

Pouyaud, Desmarais, Chenuil, Agnese, & Bonhomme. (2002). Kin cohesiveness and possible inbreeding in the mouthbrooding tilapia Sarotherodon melanotheron (Pisces Cichlidae). *Molecular Ecology, 8*(5), 803-812. doi:10.1046/j.1365-294X.1999.00632.x

Pulliam, H. R. (1973). On the advantages of flocking. *Journal of Theoretical Biology, 38*(2), 419-422. doi:10.1016/0022-5193(73)90184-7

Queiroz, H., & Magurran, A. E. (2005). Safety in numbers? Shoaling behaviour of the Amazonian red-bellied piranha. *Biology Letters, 1*(2), 155-157.

Quinn, T. P., & Busack, C. A. (1985). Chemosensory recognition of siblings in juvenile coho salmon (Oncorhynchus kisutch). *Animal Behaviour, 33*(1), 51-56.

R Core Team. (2017). R: A Language and Environment for Statistical Computing. (Version R.version 3.3.3 (Another Canoe).). Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org.

Radford, C. A., Stanley, J. A., Simpson, S. D., & Jeffs, A. G. (2011). Juvenile coral reef fish use sound to locate habitats. *Coral Reefs, 30*(2), 295-305. doi:10.1007/s00338-010-0710-6

Ranta, E., & Kaitala, V. (1991). School size affects individual feeding success in three-spined sticklebacks (Gastevosteus aculeatus L.). *Journal of Fish Biology, 39*(5), 733-737. doi:10.1111/j.1095-8649.1991.tb04402.x

Rasband, W. S. (2017). ImageJ1. Retrieved from https://imagej.net/ImageJ1

Reichard, M., Polačik, M., Blažek, R., & Vrtílek, M. (2014). Female bias in the adult sex ratio of African annual fishes: interspecific differences, seasonal trends and environmental predictors. *Evolutionary Ecology, 28*(6), 1105-1120. doi:10.1007/s10682-014-9732-9

Reichard, M., Polačik, M., & Sedláček, O. (2009). Distribution, colour polymorphism and habitat use of the African killifish Nothobranchius furzeri, the vertebrate with the shortest life span. *Journal of Fish Biology, 74*(1), 198-212. doi:10.1111/j.1095-8649.2008.02129.x

Reyes Blengini, F., Tassino, B., & Passos, C. (2018). Females of the annual killifish Austrolebias reicherti (Cyprinodontiformes: Rivulidae) recognize conspecific mates based upon chemical cues. *Behavioural Processes, 155*, 33-37. doi:https://doi.org/10.1016/j.beproc.2017.08.007

Richards, E. L., van Oosterhout, C., & Cable, J. (2010). Sex-specific differences in shoaling affects parasite transmission in guppies. *PLoS One, 5*(10), e13285. doi:10.1371/journal.pone.0013285

Riley, W. D. (2007). Seasonal downstream movements of juvenile Atlantic salmon, Salmo salar L., with evidence of solitary migration of smolts. *Aquaculture, 273*(2), 194-199. doi:https://doi.org/10.1016/j.aquaculture.2007.10.022

Rodgers, G. M., Kelley, J. L., & Morrell, L. J. (2010). Colour change and assortment in the western rainbowfish. *Animal Behaviour, 79*(5), 1025-1030. doi:https://doi.org/10.1016/j.anbehav.2010.01.017

Rothman, R. J., & Mech, L. D. (1979). Scent-marking in lone wolves and newly formed pairs. *Animal Behaviour, 27*, 750-760. doi:https://doi.org/10.1016/0003-3472(79)90010-1

Ruhl, N., & McRobert, S. P. (2005). The effect of sex and shoal size on shoaling behaviour in Danio rerio. *Journal of Fish Biology, 67*(5), 1318-1326. doi:doi:10.1111/j.0022-1112.2005.00826.x

Rybak, F., Sureau, G., & Aubin, T. (2002). Functional coupling of acoustic and chemical signals in the courtship behaviour of the male Drosophila melanogaster. *Proceedings of the Royal Society of London B: Biological Sciences, 269*(1492), 695-701.

Schielzeth, H. (2010). Simple means to improve the interpretability of regression coefficients. *Methods in Ecology and Evolution, 1*(2), 103-113.

Searby, A., & Jouventin, P. (2003). Mother-lamb acoustic recognition in sheep: a frequency coding. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 270*(1526), 1765.

Searcy, S. P., & Sponaugle, S. U. (2001). Selective mortality during the larval - juvenile transition in two coral reef fishes. *Ecology, 82*(9), 2452-2470. doi:10.1890/0012-9658(2001)082[2452:SMDTLJ]2.0.CO;2

Shaw, E. (1978). Schooling fishes: The school, a truly egalitarian form of organization in which all members of the group are alike in influence, offers substantial benefits to its participants. *American Scientist, 66*(2), 166-175.

Sherman, P., Reeve, H., & Pfennig, D. (1997). Recognition Systems. In J. Krebs & N. Davies (Eds.), *Behavioural Ecology, 4th Edition* (pp. 69-96): Oxford: Blackwell Science.

Slobodkin, L. B., & Fishelson, L. (1974). The effect of the cleaner-fish Labroides dimidiatus on the point diversity of fishes on the reef front at Eilat. *The American Naturalist, 108*(961), 369-376. doi:10.1086/282914

Smithson, M., & Verkuilen, J. (2006). A better lemon squeezer? Maximum-likelihood regression with beta-distributed dependent variables. *Psychological methods, 11*(1), 54.

Snekser, J. L., McRobert, S. P., Murphy, C. E., & Clotfelter, E. D. (2006). Aggregation behavior in wildtype and transgenic zebrafish. *Ethology, 112*(2), 181-187. doi:doi:10.1111/j.1439-0310.2006.01139.x

Spence, R., & Smith, C. (2007). The role of early learning in determining shoaling preferences based on visual cues in the zebrafish, Danio rerio. *Ethology, 113*(1), 62-67. doi:doi:10.1111/j.1439-0310.2006.01295.x

Stensland, E., Angerbjörn, A., & Berggren, P. (2003). Mixed species groups in mammals. *Mammal Review, 33*(3-4), 205-223.

Stratmann, A., & Taborsky, B. (2013). Antipredator defences of young are independently determined by genetic inheritance, maternal effects and own early experience in mouthbrooding cichlids. *Functional Ecology, 28*(4), 944-953. doi:10.1111/1365-2435.12224

Stumbo, A. D., James, C. T., Goater, C. P., & Wisenden, B. D. (2012). Shoaling as an antiparasite defence in minnows (Pimephales promelas) exposed to trematode cercariae. *Journal of Animal Ecology, 81*(6), 1319-1326. doi:10.1111/j.1365-2656.2012.02012.x

Swaney, W., Kendal, J., Capon, H., Brown, C., & Laland, K. N. (2001). Familiarity facilitates social learning of foraging behaviour in the guppy. *Animal Behaviour, 62*(3), 591-598. doi:https://doi.org/10.1006/anbe.2001.1788

Terzibasi, E., Lefrançois, C., Domenici, P., Hartmann, N., Graf, M., & Cellerino, A. (2009). Effects of dietary restriction on mortality and age-related phenotypes in the short-lived fish Nothobranchius furzeri. *Aging Cell, 8*(2), 88-99. doi:10.1111/j.1474-9726.2009.00455.x

Terzibasi, E., Valenzano, D. R., & Cellerino, A. (2007). The short-lived fish Nothobranchius furzeri as a new model system for aging studies. *Experimental Gerontology, 42*(1), 81-89. doi:https://doi.org/10.1016/j.exger.2006.06.039

Thünken, T., Bakker, T. C. M., Baldauf, S. A., & Kullmann, H. (2007). Active inbreeding in a cichlid fish and its adaptive significance. *Current Biology, 17*(3), 225-229. doi:https://doi.org/10.1016/j.cub.2006.11.053

Thünken, T., Hesse, S., Bakker, T. C. M., & Baldauf, S. A. (2016). Benefits of kin shoaling in a cichlid fish: familiar and related juveniles show better growth. *Behavioral Ecology, 27*(2), 419-425. doi:10.1093/beheco/arv166

Tibbetts, E. A. (2002). Visual signals of individual identity in the wasp Polistes fuscatus. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 269*(1499), 1423.

Tibbetts, E. A., & Dale, J. (2007). Individual recognition: it is good to be different. *Trends Ecol Evol, 22*(10), 529-537. doi:https://doi.org/10.1016/j.tree.2007.09.001

Utne-Palm, A. C., & Hart , P. (2003). The effects of familiarity on competitive interactions between threespined sticklebacks. *Oikos, 91*(2), 225-232. doi:10.1034/j.1600-0706.2000.910203.x

Valenzano Dario, R., Terzibasi, E., Cattaneo, A., Domenici, L., & Cellerino, A. (2006). Temperature affects longevity and age-related locomotor and cognitive decay in the short-lived fish Nothobranchius furzeri. *Aging Cell, 5*(3), 275-278. doi:10.1111/j.1474-9726.2006.00212.x

Viscido, S. V., Parrish, J. K., & Grünbaum, D. (2004). Individual behavior and emergent properties of fish schools: a comparison of observation and theory. *Marine Ecology Progress Series, 273*, 239-250.

Viscido, S. V., & Wethey, D. S. (2002). Quantitative analysis of fiddler crab flock movement: evidence for 'selfish herd'behaviour. *Animal Behaviour, 63*(4), 735-741.

Vrtílek, M., Žák, J., Polačik, M., Blažek, R., & Reichard, M. (2018). Longitudinal demographic study of wild populations of African annual killifish. *Scientific Reports, 8*(1), 4774. doi:10.1038/s41598-018-22878-6

Vrtílek, M., Žák, J., Pšenička, M., & Reichard, M. (2018). Extremely rapid maturation of a wild African annual fish. *Current Biology, 28*(15), R822-R824. doi:https://doi.org/10.1016/j.cub.2018.06.031

Ward, A., & Hart, P. (2003). The effects of kin and familiarity on interactions between fish. *Fish and Fisheries, 4*(4), 348-358. doi:doi:10.1046/j.1467-2979.2003.00135.x

Ward, A., & Webster, M. (2016). *Sociality: the behaviour of group-living animals*: Springer.

Ward, A., Webster, M., & Hart, P. (2007). Social recognition in wild fish populations. *Proceedings of the Royal Society of London B: Biological Sciences, 274*(1613), 1071-1077.

Ward, A. J. W., Botham, M. S., Hoare, D. J., James, R., Broom, M., Godin, J.-G. J., & Krause, J. (2002). Association patterns and shoal fidelity in the three–spined stickleback. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 269*(1508), 2451-2455.

Ward, P. (1965). Feeding ecology of the black-faced dioch quelea quelea in Nigeria. *Ibis, 107*(2), 173-214. doi:doi:10.1111/j.1474-919X.1965.tb07296.x

Ward, P., & Zahavi, A. (1973). The importance of certain assemblages of birds as "information-centres" for food-finding. *Ibis, 115*(4), 517-534.

Webster, M. M., & Laland, K. N. (2017). Social information use and social learning in non-grouping fishes. *Behavioral Ecology, 28*(6), 1547-1552. doi:10.1093/beheco/arx121

Wedekind, C., Seebeck, T., Bettens, F., & Paepke, A. J. (1995). MHC-dependent mate preferences in humans. *Proc. R. Soc. Lond. B, 260*(1359), 245-249.

Wiley, R. H. (1978). The lek mating system of the Sage Grouse. *Scientific American, 238*(5), 114-125.

Wilson, E. O., & Hölldobler, B. (2005). Eusociality: origin and consequences. *Proceedings of the National Academy of Sciences, 102*(38), 13367-13371.

Wolf, N. G. (1987). Schooling tendency and foraging benefit in the ocean surgeonfish. *Behavioral Ecology and Sociobiology, 21*(1), 59-63.

Wright, D., & Krause, J. (2006). Repeated measures of shoaling tendency in zebrafish (Danio rerio) and other small teleost fishes. *Nature Protocols., 1*, 1828-1831. doi:10.1038/nprot.2006.287

Wright, J., Stone, R. E., & Brown, N. (2003). Communal roosts as structured information centres in the raven, Corvus corax. *Journal of Animal Ecology, 72*(6), 1003-1014. doi:doi:10.1046/j.1365-2656.2003.00771.x